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BIOGEOGRAPHY AND PHYLOGENY OF *CARDAMINE* (BRASSICACEAE)[1]

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ABSTRACT

The biogeography and phylogeny of *Cardamine* L. were inferred based on sequences of the nuclear ribosomal ITS regions and the plastid *trnL* intron and *trnL-F* spacer regions. This genus is one of the largest and polyploid-rich genera of the Brassicaceae and has its center of diversity in Eurasia. Species were included from all populated continents, representing all sections except two monotypic ones. The results support a hypothesis of recent and rapid speciation in the genus. The traditional sectional classification was not supported. We found evidence for several extremely long-distance dispersal events. Colonization of the Southern Hemisphere and the Arctic has occurred repeatedly; we identified at least three phylogenetically distinct Arctic lineages, two distinct Oceanian lineages, and four distinct South American lineages. Polyploidization has occurred independently many times during the evolution of *Cardamine*. Recent divergence combined with widespread polyploidization offer an explanation for the complex taxonomy of the genus.

Key words: Arctic, biogeography, Brassicaceae, *Cardamine*, phylogeny, polyploidization.

Cardamine L. is a taxonomically complex, cosmopolitan genus with at least 160 to 200 Arctic, alpine, and boreal species, and is one of the most species-rich genera of the Brassicaceae (Sjöstedt, 1975; Hewson, 1982; Al-Shehbaz, 1988; Webb et al., 1988; Al-Shehbaz et al., 2006). The number of species accepted varies considerably among different authors, illustrating the notorious taxonomic complexity of this genus. The center of diversity is clearly situated in Eurasia; according to conservative estimates (mainly based on Al-Shehbaz, 1988), approximately 95 species are Eurasian (ca. 48 of which are in China and ca. 25 in Europe including the Caucasus). There are 43 species in North and Central America (Al-Shehbaz, pers. comm.), and, of these, at least nine species extend into Arctic areas. There are fewer native species in the Southern Hemisphere: 20 in South America (Al-Shehbaz, pers. comm.), 10 in Australia and New

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Zealand, four in New Guinea, and three in Africa. Some species are invasive cosmopolitan weeds, such as *C. hirsuta* L., *C. impatiens* L., *C. flexuosa* With., and *C. parviflora* L.

In O. E. Schulz's (1903) monograph of the genus, 116 species were accepted and classified into 12 sections. Schulz (1936) later extended his account to include ca. 130 species in 13 sections (Table 1). Of special interest in a biogeographic context are the three largest sections of *Cardamine*: section *Cardamine* L. (Schulz's *Eucardamine* Godr.), section *Dentaria* L., and section *Cardaminella* Prantl. In Schulz's treatments (1903, 1936), section *Cardamine* includes ca. 74 species, has a global distribution, and encompasses a wide range of morphological variation. His section *Dentaria* contains 16 perennial species from North America and Europe characterized by fleshy creeping rhizomes and typically large, showy flowers. Schulz's section *Cardaminella* has a highly disjunct distribution, including four species from the Arctic (including Beringia), three species from alpine areas in Europe, one species from Japan, and three species from Oceania, all of them small, cold-adapted plants. Also noteworthy are six monotypic sections (Table 1).

Many new species have been described since Schulz's (1903, 1936) revisions, but species delimitation is difficult and the total number of species in *Cardamine* remains controversial. The sectional partitioning of Schulz has been criticized by several authors for overemphasizing a few morphological characters (Al-Shehbaz, 1988; Rashid & Ohba, 1993). Previous studies in *Cardamine* have shown that some of his sections (*Cardamine*, *Dentaria*, *Macrocarpus* O. E. Schulz, *Macrophyllum* O. E. Schulz, and *Papyrophyllum* O. E. Schulz) are not monophyletic (Rashid & Ohba, 1993; Franzke et al., 1998; Sweeney & Price, 2000; Bleeker et al., 2002). Several species groups in *Cardamine* have been studied quite extensively based on molecular as well as cytological and morphological data. The *C. pratensis* L. complex, for example, has a history of recurrent polyploidization events and dispersals over relatively long distances (Marhold & Ancev, 1999; Franzke & Hurka, 2000; Marhold et al., 2002, 2004; Lihova et al., 2004; Marhold & Lihova, 2006). However, no genus-wide molecular analysis of *Cardamine* has been performed so far.

Cardamine is probably a fairly young genus. Molecular data indicate that a clade comprising the genera *Barbarea* W. T. Aiton, *Armoracia* G. Gaertn., B. Mey. & Scherb., and *Rorippa* Scop. is sister to a *Cardamine–Nasturtium* W. T. Aiton clade (Franzke et al., 1998; Yang et al., 1999; Koch et al., 2001). *Rorippa* pollen is first found in sediments from the Pliocene (2.5–5 Ma) (Mai, 1995). Koch et al. (2000) used this time span to estimate that the lineages that gave rise to *Cardamine* and *Barbarea* diverged 6.0 Ma. This was suggested to be an underestimate by Heads (2005), as he doubted the dating of the pollen. However, using the nuclear data set of Koch et al. (2000), Haubold and Wiehe (2001) performed a more thorough study under various evolutionary rate assumptions, all resulting in a divergence time of 6.2 Ma.

Most species of *Cardamine* are polyploid, and up to five basic chromosome numbers have been suggested (Al-Shehbaz, 1988). The most probable basic number for the majority of species is $x = 8$ (Kucera et al., 2005). For some species, such as the Beringian taxa in section *Cardaminella*, the most probable basic number is $x = 7$ (Elven et al., 2006). Diploids are only known with $2n = 16$, and the highest recorded number is $2n = 32x = 256$ (*C. concatenata* (Michx.) O. Schwartz and *C. diphylla* (Michx.) Alph. Wood; Harriman, 1965; Kucera et al., 2005; Warwick & Al-Shehbaz, 2006).

The seeds of *Cardamine* are ejected by curling of the silique walls, a typical short-distance mode of dispersal (Kimata, 1983). *Cardamine* is nevertheless found on all continents except Antarctica. Under moist conditions, the seeds can become mucilaginous and adhere to animals (Al-Shehbaz, 1988). As the majority of *Cardamine* species occur in moist habitats, this may be a common mode of dispersal, also across vast areas via birds. Dispersal between Eurasia and North America may have occurred stepwise via the Tertiary Beringian land bridge that existed until 5.4–5.5 Ma (Marincovich & Gladenkov, 1999, 2001; Gladenkov et al., 2002), but dispersal over longer distances must have occurred between these *Cardamine*-rich continents and Oceania, South America, and Africa.

In this paper, we particularly address the occurrence of such long-distance colonization events, including the colonization of the biogeographically young Arctic region. Among the ca. nine species of *Cardamine* occurring in the Arctic, two (*C. bellidifolia* L. and *C. pratensis* s.l.) have complete circumpolar distributions, and seven are restricted to the Beringian region (*C. blaisdellii* Eastw., *C. digitata* Richardson, *C. purpurea* Cham. & Schltdl., *C. pedata* Regel & Tiling, *C. microphylla* Adams, *C. victoris* N. Busch, and *C. sphenophylla* Jurtzev). The current Arctic tundra replaced a more or less continuous forest following the climatic shift in the late Tertiary (Lafontaine & Wood, 1988; Bennike & Böcher, 1990; Matthews & Ovenden, 1990; Murray, 1995; Lear et al., 2000). Murray (1995) suggested that the Arctic flora of today is composed of a mixture of survivors from the Arctic Tertiary forest, Pleistocene immigrants from various mountain areas, and in situ–evolved Pleistocene taxa.

Here we attempt to reconstruct the phylogeny of *Cardamine* based on extensive, genus-wide species sampling and sequencing of several DNA regions (using the nuclear ribosomal ITS regions and the plastid *trnL* intron and *trnL-F* spacer regions in the final analysis). In particular, we address the infrageneric classification of *Cardamine*, especially in light of Schulz (1903, 1936), and what is already known from Franzke et al. (1998), Sweeney and Price (2000), and Bleeker et al. (2002). We also examine biogeographic patterns in this widespread genus, and particularly address dispersals and source areas for colonization of the Arctic and Southern Hemisphere.

MATERIALS AND METHODS

Fresh leaf material was sampled and dried in silica gel in the field. Vouchers are deposited in the herbaria at the Natural History Museum, University of Oslo (O), and the University of Osnabrück (OSBU). Leaf material was also sampled from herbarium specimens in ALA, CAN, CANB, DAO, HBG, LE, O, OSBU, OSC, S, UPS, and WU (Appendix 1). Species of *Cardamine* representing all continents where *Cardamine* occurs and 10 of the 13 sections described by Schulz (1903, 1936) were included: exceptions are the monotypic sections *Giraldiella* O. E. Schulz, *Lygophyllum* O. E. Schulz, and *Spirobolus* O. E. Schulz (Table 1).

DNA was extracted using the DNeasy Plant Mini Kit or DNeasy Plant 96 Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. We initially tested several DNA regions for a subset of species. The mitochondrial *nad6* gene, the nuclear 5S non-transcribed spacer region, and the plastid regions *trnT-trnL* spacer, *psbA-trnH* spacer, *trnS-trnG* spacer, and *ndhF* gene were tested but found either not variable enough (*nad6*), too variable and difficult to align (the 5S non-transcribed spacer and *psbA-trnH*), or difficult to sequence (*ndhF*, *trnS-trnG*, and *trnT-trnL*). The only useful regions were found to be ITS and the plastid *trnL* intron and *trnL-F* spacer regions.

PCR amplification of ITS was performed with primers ITS-4 and ITS-5 (White et al., 1990) using 30 cycles of 45 sec. at 94°C (first cycle, 5 min.), 45 sec. at 55°C, and 90 sec. at 72°C (last cycle, 10 min.). The *trnL* intron was amplified with the primers c and d, and the *trnL-trnF* intergenic spacer region with the primers e and f (Taberlet et al., 1991) using 30 cycles of 30 sec. at 94°C (first cycle, 5 min.), 30 sec. at 55°C, and 90 sec. at 72°C (last cycle, 10 min.). PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, Ohio, U.S.A.) before cycle sequencing with BigDye (Applied Biosystems, Foster City, California, U.S.A.) using 25 cycles of 10 sec. at 96°C, 5 sec. at 50°C, and 240 sec. at 60°C.

Sequences were edited in Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan, U.S.A.), and ambiguous positions were coded according to the International Union of Pure and Applied Chemistry (IUPAC) standards. Sequences were translated to RNA and analyzed in RNAfold (Hofacker et al., 1994) and MARNA (Siebert & Backofen, 2005) to detect secondary structure and to ensure that conserved stem (helix) regions were aligned correctly. The sequences were subsequently aligned manually in BioEdit (Hall, 1999). The three regions corresponding to the hairpin loop in helix III in ITS-2 and to loops in

Table 1. Sectional classification, geographic distribution, and number of species according to Schulz (1936). The number of species in some of the sections has increased after 1936.

Section	Abbreviation (cf. Fig. 1)	No. of spp.	Geographic distribution
I. *Dentaria* L.	Dent	16	Eurasia and Atlantic North America
II. *Eutreptophyllum* O. E. Schulz	Eutr	2	Pacific North America
III. *Sphaerotorrhiza* O. E. Schulz	Sphae	1	Siberia
IV. *Coriophyllum* O. E. Schulz	Corio	1	Middle Europe
V. *Giraldiella* O. E. Schulz[a]	Girar	1	China
VI. *Macrophyllum* O. E. Schulz	Mac-ph	7	Asia and North America
VII. *Lygophyllum* O. E. Schulz[a]	Lygo	1	Himalaya
VIII. *Papyrophyllum* O. E. Schulz	Papyro	8	tropical mountains
IX. *Eucardamine* Godr.			
– *Cardamine* L.	Card	ca. 74	cosmopolitan
X. *Cardaminella* Prantl	C-nella	12	cold areas all over the world
XI. *Pteroneurum* (DC.) Nyman	Ptero	5	East Mediterranean region
XII. *Spirobolus* O. E. Schulz[a]	Spiro	1	Mediterranean region
XIII. *Macrocarpus* O. E. Schulz	Mac-ca	1	South America

[a] Not included in our study.

helices III and IV in ITS-1 could not be unambiguously aligned, and these regions were therefore excluded from the final matrix. For the *trnL-F* spacer region, non-homologous pseudogene replications were excluded from the matrix prior to all analyses (Koch et al., 2005). In addition, plastid and nuclear sequences of *Cardamine* and related genera from GenBank were imported into the matrices and aligned manually, resulting in a data set including a total of 111 species of *Cardamine* (Appendix 1). Several genera were tested as outgroup, but *Rorippa* was chosen in the end as the most suitable alternative because it was the closest related genus among the available genera.

Parsimony analyses were performed in Tree Analysis Using New Technology (TNT) (Goloboff et al., 2003) with potential parsimony informative gaps coded as present/absent (Simmons & Ochoterena, 2000). Heuristic searches were performed with 1000 random addition sequences and tree bisection-reconnection (TBR) branch swapping, saving 10 trees per replication. The resulting trees were swapped on with TBR saving up to 100,000 trees. Collapsing rule was set to minimum length = 0. Random seed was set to "time." Goodness of fit was calculated using consistency index (CI), retention index (RI), and rescaled consistency index (RC) (Kluge & Farris, 1969; Farris, 1989). Bremer support (Bremer, 1994) was calculated by producing 120,000 trees that were up to 12 steps longer, starting with saving 10,000 trees one step longer, and successively saving 10,000 trees of up to one step longer in 11 steps. Jackknife (Farris et al., 1996) and bootstrap (Felsenstein, 1985) resampling were performed with 1000 replicates (10 random entry orders and 10 trees saved in each repetition) and collapsing rule = TBR. Jackknifing was performed with 36% deletion. Bootstrap and jackknife were performed with a cut-off value of 50% and absolute frequencies as output. Implied weighting (Goloboff, 1993) was performed with K = 1, 3, 6, 8, 20, and 50. In addition to the analysis of all taxa, separate analyses were performed on diploid taxa, tetraploid taxa, and diploid and tetraploid taxa together.

A Bayesian analysis was performed on the ITS data set in MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with the model general time reversible (GTR) + gamma provided by MrAIC (Nylander, 2004). The analysis was run with the default settings in MrBayes, random starting trees and run for 3,000,000 generations with sampling of Markov chains for each 100th generation. The first 25% of the trees were discarded as "burn-in" samples. A Bayesian analysis was also performed on the plastid data set, but did not provide any additional information and was thus excluded.

Results

The final aligned ITS matrix included 629 characters, of which 188 were parsimony informative (186 when excluding the outgroup). There were four potential parsimony informative coded gaps in the data matrix (2 gaps of length 1 bp and 2 gaps of length 2 bp). The most parsimonious trees (MPTs) inferred from the ITS data set were 749 steps long with CI = 0.530, RI = 0.692, and RC = 0.367; one of them, as well as the strict consensus tree, is presented in Figure 1. The separate parsimony analyses of diploids, tetraploids, and diploids and tetraploids together did not give better resolution or conflicting topologies (result not shown). Using implied weighing did not give better resolution or conflicting topologies (result not shown). The Bayesian analysis of the ITS data set is presented in Figure 2.

We partitioned the ITS trees into nine operational groups to simplify presentation (marked A–J in Figs. 1, 2).

Group A was supported by a posterior probability (PP) of 0.96 and Bremer support (BR) = 1. This group included only diploid ($2n = 16$) European species (with *Cardamine bellidifolia* extending into the circumpolar area), four of them belonging to section *Cardaminella* (*C. alpina* Willd., *C. bellidifolia*, *C. plumieri* Vill., and *C. resedifolia* L.) and one (*C. carnosa* Waldst. & Kit.) to section *Pteroneurum* (DC.) Nyman.

Group B was supported by BR = 1 and comprised the East Asian *Cardamine tenuifolia* (Ledeb.) Turcz. of the monotypic section *Sphaerotorrhiza* O. E. Schulz and the African *C. trichocarpa* Hochst. ex A. Rich. of section *Cardamine*.

Group C was supported by BR = 1 and included Eurasian and North American taxa. North American high polyploids ($2n = 12x = 96$ to $2n = 32x = 256$) of section *Dentaria* (*Cardamine angustata* O. E. Schulz, *C. concatenata*, *C. dissecta* (Leavenw.) Al-Shehbaz, and *C. diphylla*) formed a clade supported by jackknife (JK) = 93%, bootstrap (BS) = 88%, BR = 5, and PP = 1.0. The European species of section *Dentaria* (*C. bipinnata* O. E. Schulz, *C. bulbifera* Crantz, *C. glanduligera* O. Schwarz, *C. abchasica* Govaerts, and *C. quinquefolia* (M. Bieb.) Benth. & Hook. f. ex Schmalh.) together with the Asian diploid *C. leucantha* O. E. Schulz of section *Macrophyllum* formed a clade supported by JK = 91%, BS = 87%, BR = 4, and PP = 1.0.

Group D was supported by BR = 1 and included the East Asian polyploids *Cardamine macrophylla* Willd. and *C. tangutorum* O. E. Schulz and the European diploid *C. trifolia* L., belonging to three different sections (*Macrophyllum*, *Dentaria*, and *Coriophyllum* O. E. Schulz, respectively).

Group E was supported by BR – 2 and PP – 1.0. This group included the European *Cardamine waldsteinii* Dyer of section *Dentaria* in addition to two well-supported clades with East Asian and North American taxa, respectively. The East Asian clade (JK – 98%, BS – 97%, BR – 5, and PP – 1.0) included members of sections *Cardaminella* (*C. nipponica* Franch. & Sav.) and *Cardamine* (*C. microzyga* O. E. Schulz). The North American clade (JK – 100%, BS – 99%, BR – 12, and PP – 1.0) also included the cosmopolitan weed *C. hirsuta* of section *Cardamine*.

Group F was supported by BR – 1 and PP – 1.0. This group included species of section *Cardamine* from South America, East Asia, and Africa.

Group G was supported by BR – 1 and PP – 0.98. This group included Eurasian, African, and South American taxa. Most Asian species grouped together, containing members of both sections *Cardamine* and *Macrophyllum*. Notably, the accessions of *C. scutata* Thunb. from Japan and Taiwan did not group together. The South American *C. ovata* Benth. grouped with African and South American *C. africana* L., both belonging to section *Papyrophyllum*. The chromosome numbers in this group spanned from $2n – 16$ (diploid) to $2n – 56$.

Group H was supported by JK – 65%, BS – 50%, BR – 4, and PP – 1.0. This group included a specimen from New Guinea referred to as *Cardamine africana*; it did not group with the other *C. africana* accessions (group G) and most likely represents a different taxon. All four species known from New Guinea belonged to this group, which also included European species of section *Dentaria* (*C. heptaphylla* (Vill.) O. E. Schulz, *C. kitaibelii* Bech., and *C. pentaphyllos* (L.) Crantz).

Group I was supported by BR – 1 and PP – 0.94. This group included the *Cardamine pratensis* species group and its closely related European species, which also formed a clade in the plastid analysis (Fig. 3; JK – 81%, BS – 81%, BR – 3). As this group has been extensively studied earlier (Franzke et al., 1998; Franzke & Hurka, 2000; Lihova & Marhold, 2003) and our analyses supported their findings without adding new information, we pruned several taxa of this complex from our final analyses and retained only four species related to *C. pratensis* to simplify this presentation (*C. acris* Griseb., *C. flaccida* Cham. & Schltdl., *C. pratensis*, and *C. tenera* S. G. Gmel. ex C. A. Mey.).

Group J had no support and was only present in the MPTs and a combinable components consensus tree, but included several supported subgroups. This group included most of the Beringian taxa, all of the Australian and New Zealand taxa, and many North American taxa in addition to one species from South America and East Asia, respectively. The Australian and New Zealand taxa, *Cardamine debilis* Banks ex DC., *C. lacustris* (Garn.-Jones & P. N. Johnson) Heenan, *C. lilacina* Hook., and *C. paucijuga* Turcz., constituted a monophyletic group (JK – 55%, BS – 57%, BR – 1, and PP – 0.98) with the inclusion of the South American *C. glacialis* (G. Forst.) DC. and the amphi-Beringian/Pacific *C. umbellata* Greene. The ITS data set was inconclusive about the monophyly of the remaining Beringian species (*C. blaisdellii*, *C. digitata*, *C. purpurea*, *C. pedata*, *C. microphylla*, *C. victoris*, and *C. sphenophylla*). However, most of the MPTs supported the Beringian species as a monophyletic group with North American taxa as sister groups. The remaining trees supported the Beringian species as two separate groups, but both of them with North American species as sister groups. This led to the collapse of these branches in the strict consensus tree and the resampling analyses.

The South American species appeared scattered in the tree. *Cardamine glacialis* was most closely related to the taxa from Oceania in group J, *C. bonariensis* Pers. and *C. flaccida* close to or nested within the *C. pratensis* group in group I, and *C. ecuadorensis* Hieron. and *C. rhizomata* Rollins were resolved as a sister group to *C. griffithii* Hook. f. & Thomson in group F.

The three African species did not form a monophyletic group. *Cardamine trichocarpa* was found in group B, while *C. obliqua* Hochst. ex. A. Rich. was sister to *C. lihengiana* Al-Shehbaz in group F with BR – 2 and PP – 0.96. A specimen from South America referred to *C. africana* was more closely related to South American *C. ovata* than to a *C. africana* specimen from Kilimanjaro.

The Oceanian taxa occurred in two distinct clades. In group H (JK = 65%, BS = 50%, BR – 4, and PP – 1.0), three European species were nested among four species from New Guinea (*Cardamine* sp. aff. *africana* L., *C. altigena* Schltr. ex O. E. Schulz, *C. keysseri* O. E. Schulz, and *C. papuana* O. E. Schulz). In group J, four species from Australia and New Zealand (*C. paucijuga*, *C. lilacina*, *C. lacustris*, and *C. debilis*) were most closely related (JK – 54%, BS – 57%, and PP – 0.98) to the Arctic *C. umbellata* and the South American *C. glacialis*.

The Beringian/circumpolar taxa occurred in three different groups. *Cardamine pratensis* (group I) and *C. bellidifolia* (group A) had their closest relatives in Europe. In group J, there were seven Beringian species (*C. blaisdellii*, *C. digitata*, *C. purpurea*, *C. pedata*, *C. microphylla*, *C. victoris*, and *C. sphenophylla*) possibly having their closest relatives in Oceania and North America.

The aligned *trnL-F* matrix included 765 characters, 77 of which were parsimony informative (71 when

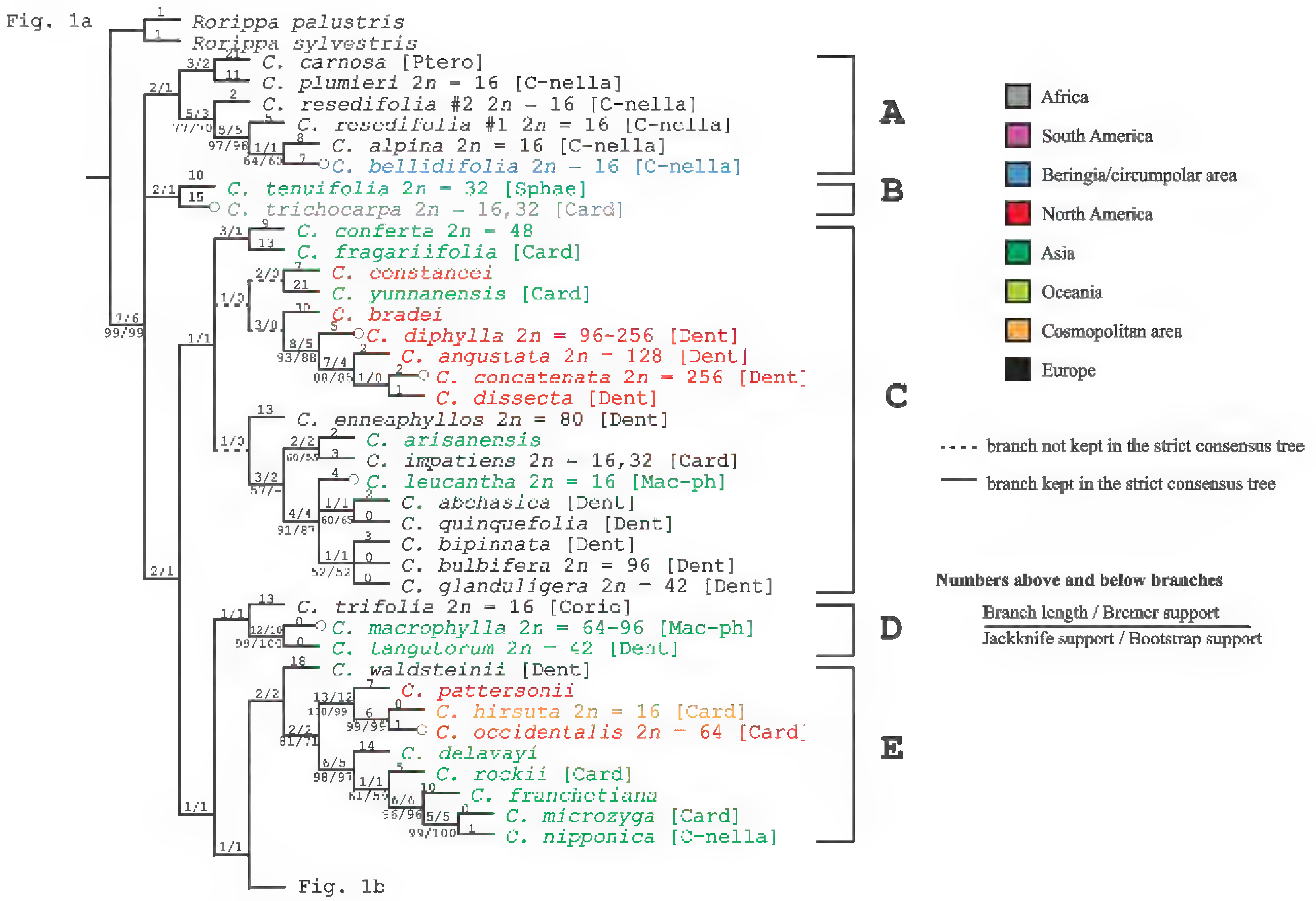
Fig. 1a
Rorippa palustris
Rorippa sylvestris
C. carnosa [Ptero]
C. plumieri 2n = 16 [C-nella]
C. resedifolia #2 2n = 16 [C-nella]
C. resedifolia #1 2n = 16 [C-nella]
C. alpina 2n = 16 [C-nella]
C. bellidifolia 2n = 16 [C-nella]
C. tenuifolia 2n = 32 [Sphae]
C. trichocarpa 2n = 16,32 [Card]
C. conferta 2n = 48
C. fragariifolia [Card]
C. constancei
C. yunnanensis [Card]
C. bradei
C. diphylla 2n = 96-256 [Dent]
C. angustata 2n = 128 [Dent]
C. concatenata 2n = 256 [Dent]
C. dissecta [Dent]
C. enneaphyllos 2n = 80 [Dent]
C. arisanensis
C. impatiens 2n = 16,32 [Card]
C. leucantha 2n = 16 [Mac-ph]
C. abchasica [Dent]
C. quinquefolia [Dent]
C. bipinnata [Dent]
C. bulbifera 2n = 96 [Dent]
C. glanduligera 2n = 42 [Dent]
C. trifolia 2n = 16 [Corio]
C. macrophylla 2n = 64-96 [Mac-ph]
C. tangutorum 2n = 42 [Dent]
C. waldsteinii [Dent]
C. pattersonii
C. hirsuta 2n = 16 [Card]
C. occidentalis 2n = 64 [Card]
C. delavayi
C. rockii [Card]
C. franchetiana
C. microzyga [Card]
C. nipponica [C-nella]
Fig. 1b
A
B
C
D
E
Africa
South America
Beringia/circumpolar area
North America
Asia
Oceania
Cosmopolitan area
Europe
branch not kept in the strict consensus tree
branch kept in the strict consensus tree
Numbers above and below branches
Branch length / Bremer support
Jackknife support / Bootstrap support

Figure 1. One of the MPTs based on the ITS data set showing branches kept in the strict consensus tree. The tree length is 749 steps and the goodness of fit values are CI = 0.530, RI = 0.692, and RC = 0.367. Numbers above branches indicate branch lengths/Bremer support. Numbers below branches indicate jackknife support/bootstrap support. To simplify presentation, multiple accessions of the same taxon are indicated with a single open circle for the terminal. Chromosome numbers are given after the species name based on previously published data summarized in Kucera et al. (2005) and are followed by the section name in brackets (cf. Table 1). Letters A–J designate groups commented on in the text.

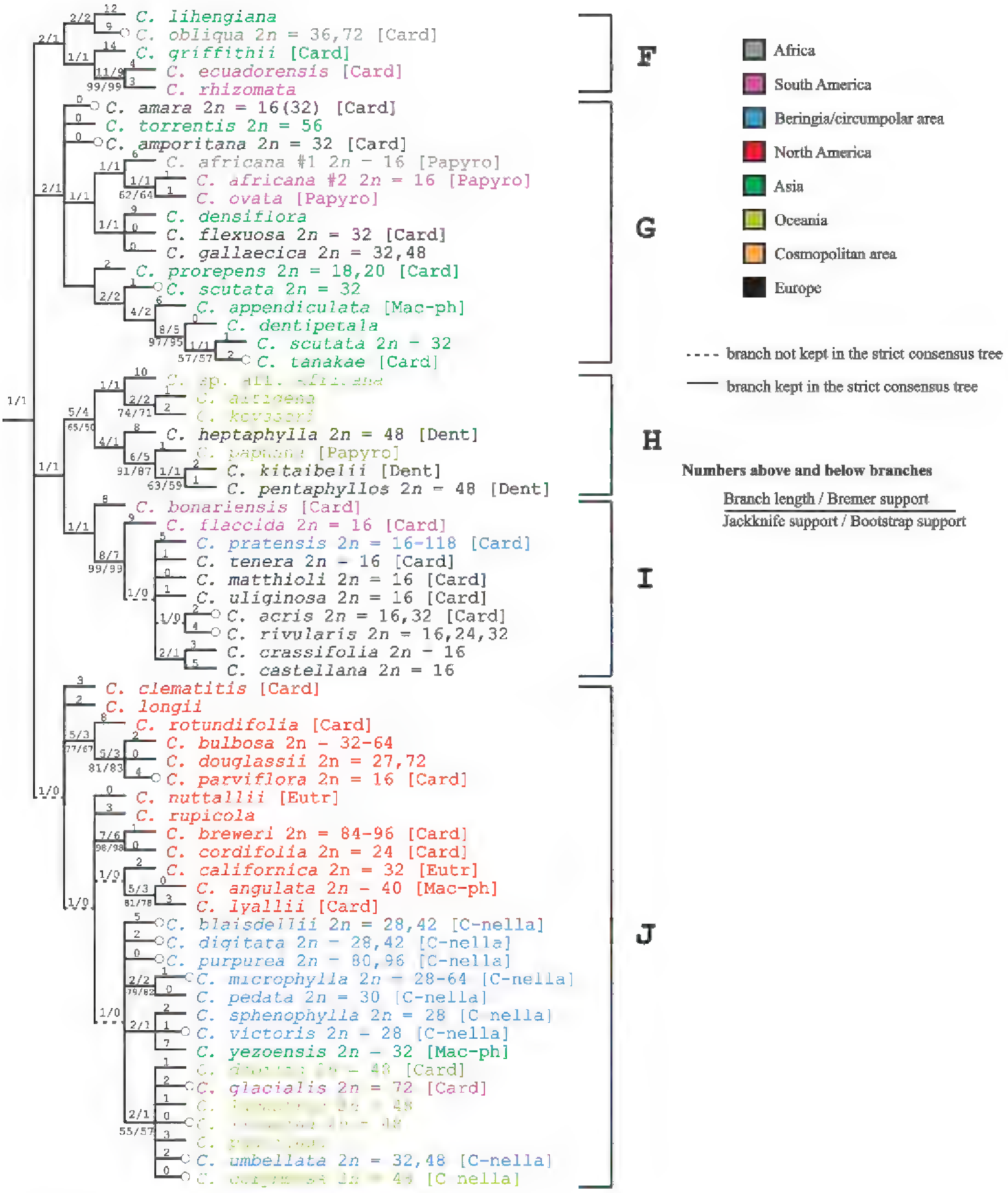
C. lihengiana
C. obliqua 2n = 36,72 [Card]
C. griffithii [Card]
C. ecuadorensis [Card]
C. rhizomata
C. amara 2n = 16(32) [Card]
C. torrentis 2n = 56
C. amporitana 2n = 32 [Card]
C. africana #1 2n = 16 [Papyro]
C. africana #2 2n = 16 [Papyro]
C. ovata [Papyro]
C. densiflora
C. flexuosa 2n = 32 [Card]
C. gallaecica 2n = 32,48
C. prorepens 2n = 18,20 [Card]
C. scutata 2n = 32
C. appendiculata [Mac-ph]
C. dentipetala
C. scutata 2n = 32
C. tanakae [Card]
C. sp. aff. africana
C. heptaphylla 2n = 48 [Dent]
[Papyro]
C. kitaibelii [Dent]
C. pentaphyllos 2n = 48 [Dent]
C. bonariensis [Card]
C. flaccida 2n = 16 [Card]
C. pratensis 2n = 16-118 [Card]
C. tenera 2n = 16 [Card]
C. matthioli 2n = 16 [Card]
C. uliginosa 2n = 16 [Card]
C. acris 2n = 16,32 [Card]
C. rivularis 2n = 16,24,32
C. crassifolia 2n = 16
C. castellana 2n = 16
C. clematitis [Card]
C. longii
C. rotundifolia [Card]
C. bulbosa 2n = 32-64
C. douglassii 2n = 27,72
C. parviflora 2n = 16 [Card]
C. nuttallii [Eutr]
C. rupicola
C. breweri 2n = 84-96 [Card]
C. cordifolia 2n = 24 [Card]
C. californica 2n = 32 [Eutr]
C. angulata 2n = 40 [Mac-ph]
C. lyallii [Card]
C. blaisdellii 2n = 28,42 [C-nella]
C. digitata 2n = 28,42 [C-nella]
C. purpurea 2n = 80,96 [C-nella]
C. microphylla 2n = 28-64 [C-nella]
C. pedata 2n = 30 [C-nella]
C. sphenophylla 2n = 28 [C nella]
C. victoris 2n = 28 [C-nella]
C. yezoensis 2n = 32 [Mac-ph]
[Card]
C. glacialis 2n = 72 [Card]
C. umbellata 2n = 32,48 [C-nella]
[C nella]
F
G
H
I
J
Africa
South America
Beringia/circumpolar area
North America
Asia
Oceania
Cosmopolitan area
Europe
branch not kept in the strict consensus tree
branch kept in the strict consensus tree
Numbers above and below branches
Branch length / Bremer support
Jackknife support / Bootstrap support

Figure 1. Continued.

excluding the outgroup). There were 11 potential parsimony informative coded gaps in the data matrix with lengths spanning from 1 bp to 11 bp. The MPTs resulting from the *trnL-F* analysis were 258 steps long with CI = 0.694, RI = 0.807, and RC = 0.560; one of them, as well as the strict consensus tree, is presented in Figure 3. In terms of initial similarity retained as synapomorphy (RI), the plastid characters were more self-congruent than the ITS characters. The analysis of the plastid data set (Fig. 3) resulted in a poorly resolved strict consensus tree, but several interesting groups were recovered in the analyses. The European diploid *Cardaminella* taxa (*Cardamine bellidifolia*, *C. alpina*, and *C. resedifolia*) constituted a monophyletic group with 91% JK support, 88% BS support, and a BR support of 2 with *C. glauca* Spreng. as sister (JK = 67%, BS = 55%, and BR = 1). There was also support (BR = 1) for monophyly of the Oceanian

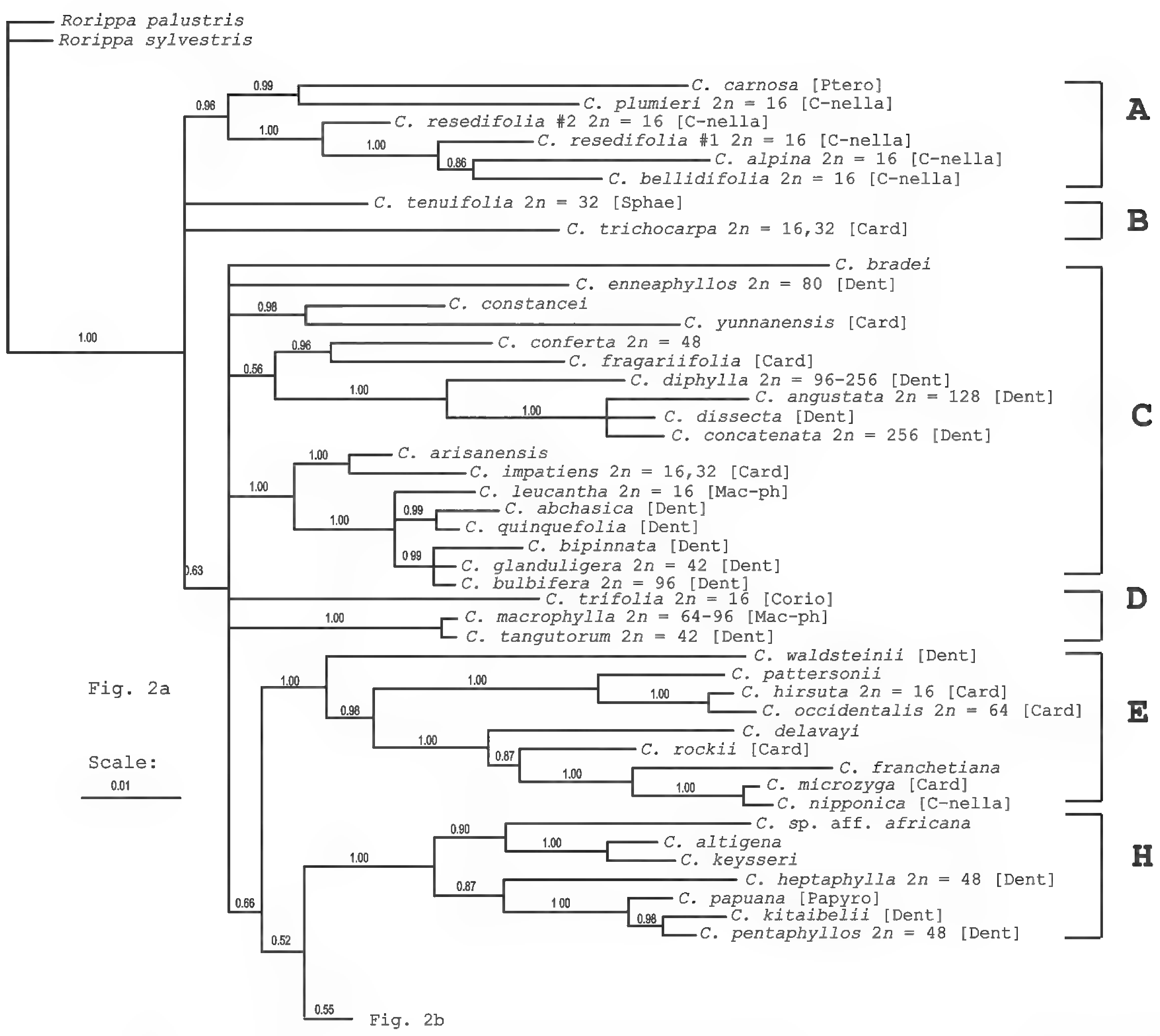
Rorippa palustris
Rorippa sylvestris
C. carnosa [Ptero]
C. plumieri 2n = 16 [C-nella]
C. resedifolia #2 2n = 16 [C-nella]
C. resedifolia #1 2n = 16 [C-nella]
C. alpina 2n = 16 [C-nella]
C. bellidifolia 2n = 16 [C-nella]
C. tenuifolia 2n = 32 [Sphae]
C. trichocarpa 2n = 16,32 [Card]
C. bradei
C. enneaphyllos 2n = 80 [Dent]
C. constancei
C. yunnanensis [Card]
C. conferta 2n = 48
C. fragariifolia [Card]
C. diphylla 2n = 96-256 [Dent]
C. angustata 2n = 128 [Dent]
C. dissecta [Dent]
C. concatenata 2n = 256 [Dent]
C. arisanensis
C. impatiens 2n = 16,32 [Card]
C. leucantha 2n = 16 [Mac-ph]
C. abchasica [Dent]
C. quinquefolia [Dent]
C. bipinnata [Dent]
C. glanduligera 2n = 42 [Dent]
C. bulbifera 2n = 96 [Dent]
C. trifolia 2n = 16 [Corio]
C. macrophylla 2n = 64-96 [Mac-ph]
C. tangutorum 2n = 42 [Dent]
C. waldsteinii [Dent]
C. pattersonii
C. hirsuta 2n = 16 [Card]
C. occidentalis 2n = 64 [Card]
C. delavayi
C. rockii [Card]
C. franchetiana
C. microzyga [Card]
C. nipponica [C-nella]
C. sp. aff. africana
C. altigena
C. keysseri
C. heptaphylla 2n = 48 [Dent]
C. papuana [Papyro]
C. kitaibelii [Dent]
C. pentaphyllos 2n = 48 [Dent]
A
B
C
D
E
H
Fig. 2a
Scale:
0.01
Fig. 2b
1.00
0.96
0.99
1.00
1.00
0.86
0.63
0.98
0.56
0.96
1.00
1.00
1.00
1.00
1.00
0.99
0.99
1.00
0.66
1.00
0.98
1.00
1.00
1.00
0.87
1.00
1.00
0.52
1.00
0.90
1.00
0.87
1.00
0.98
0.55

Figure 2. Bayesian phylogram based on the ITS data set. Numbers above branches indicate posterior probabilities. Chromosome numbers are given after the species name based on previously published data summarized in Kucera et al. (2005) and are followed by the section name in brackets (cf. Table 1). Letters A–J designate groups commented on in the text.

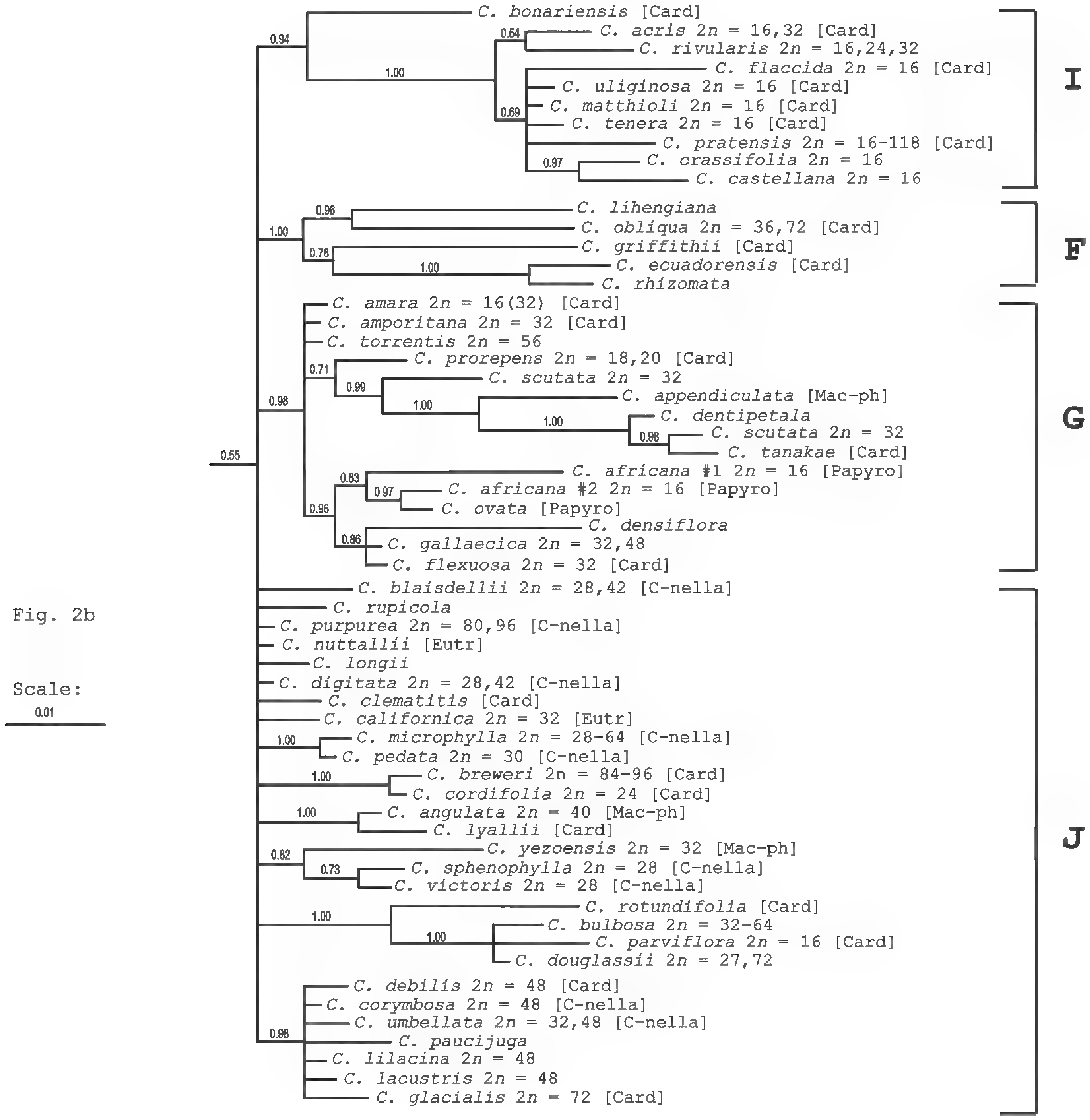
Fig. 2b
Scale:
0.01
C. bonariensis [Card]
0.94
0.54
C. acris 2n = 16,32 [Card]
C. rivularis 2n = 16,24,32
1.00
C. flaccida 2n = 16 [Card]
C. uliginosa 2n = 16 [Card]
0.69
C. matthioli 2n = 16 [Card]
C. tenera 2n = 16 [Card]
C. pratensis 2n = 16-118 [Card]
0.97
C. crassifolia 2n = 16
C. castellana 2n = 16
I
0.96
C. lihengiana
1.00
C. obliqua 2n = 36,72 [Card]
0.78
C. griffithii [Card]
1.00
C. ecuadorensis [Card]
C. rhizomata
F
C. amara 2n = 16(32) [Card]
C. amporitana 2n = 32 [Card]
C. torrentis 2n = 56
0.71
C. prorepens 2n = 18,20 [Card]
0.99
C. scutata 2n = 32
0.98
1.00
C. appendiculata [Mac-ph]
1.00
C. dentipetala
0.98
C. scutata 2n = 32
C. tanakae [Card]
0.55
0.83
C. africana #1 2n = 16 [Papyro]
0.97
C. africana #2 2n = 16 [Papyro]
C. ovata [Papyro]
0.96
C. densiflora
0.86
C. gallaecica 2n = 32,48
C. flexuosa 2n = 32 [Card]
G
C. blaisdellii 2n = 28,42 [C-nella]
C. rupicola
C. purpurea 2n = 80,96 [C-nella]
C. nuttallii [Eutr]
C. longii
C. digitata 2n = 28,42 [C-nella]
C. clematitis [Card]
C. californica 2n = 32 [Eutr]
1.00
C. microphylla 2n = 28-64 [C-nella]
C. pedata 2n = 30 [C-nella]
1.00
C. breweri 2n = 84-96 [Card]
C. cordifolia 2n = 24 [Card]
1.00
C. angulata 2n = 40 [Mac-ph]
C. lyallii [Card]
0.82
C. yezoensis 2n = 32 [Mac-ph]
0.73
C. sphenophylla 2n = 28 [C-nella]
C. victoris 2n = 28 [C-nella]
1.00
C. rotundifolia [Card]
1.00
C. bulbosa 2n = 32-64
C. parviflora 2n = 16 [Card]
C. douglassii 2n = 27,72
C. debilis 2n = 48 [Card]
C. corymbosa 2n = 48 [C-nella]
C. umbellata 2n = 32,48 [C-nella]
0.98
C. paucijuga
C. lilacina 2n = 48
C. lacustris 2n = 48
C. glacialis 2n = 72 [Card]
J

Figure 2. Continued.

Cardaminella species (*C. corymbosa* Hook. f., *C. debilis*, and *C. lilacina*) with the inclusion of the Beringian *C. victoris* and *C. umbellata*, the North American *C. cordifolia* A. Gray, and the South American *C. glacialis*. The four European species previously shown to be related to the *C. pratensis* complex (*C. pratensis*, *C. matthioli* Moretti ex Comolli, *C. penzesii* Ančev & Marhold, and *C. rivularis* Schur) formed a group supported by 81% in both JK and BS, and 3 in BR. The North American high polyploids (*C. angustata*, *C. concatenata*, *C. diphylla*, and *C. dissecta*) also formed a clade (JK = 82%, BS = 76%, and BR = 1).

DISCUSSION

RAPID DIVERSIFICATION AND WIDESPREAD POLYPLOIDIZATION

Although 29% of the characters in the ITS data set were phylogenetically informative, only a few of them were useful for resolving deeper relationships in *Cardamine*. Even though the overall RI for the ITS trees might suggest that there is substantial homoplasy in the data set, with 31% of all characters being retained as such, implied weighting did not affect the topology or improve the resolution of the deeper relationships as one might expect when reducing the effect of homoplastic characters. The most likely

Fig. 3a

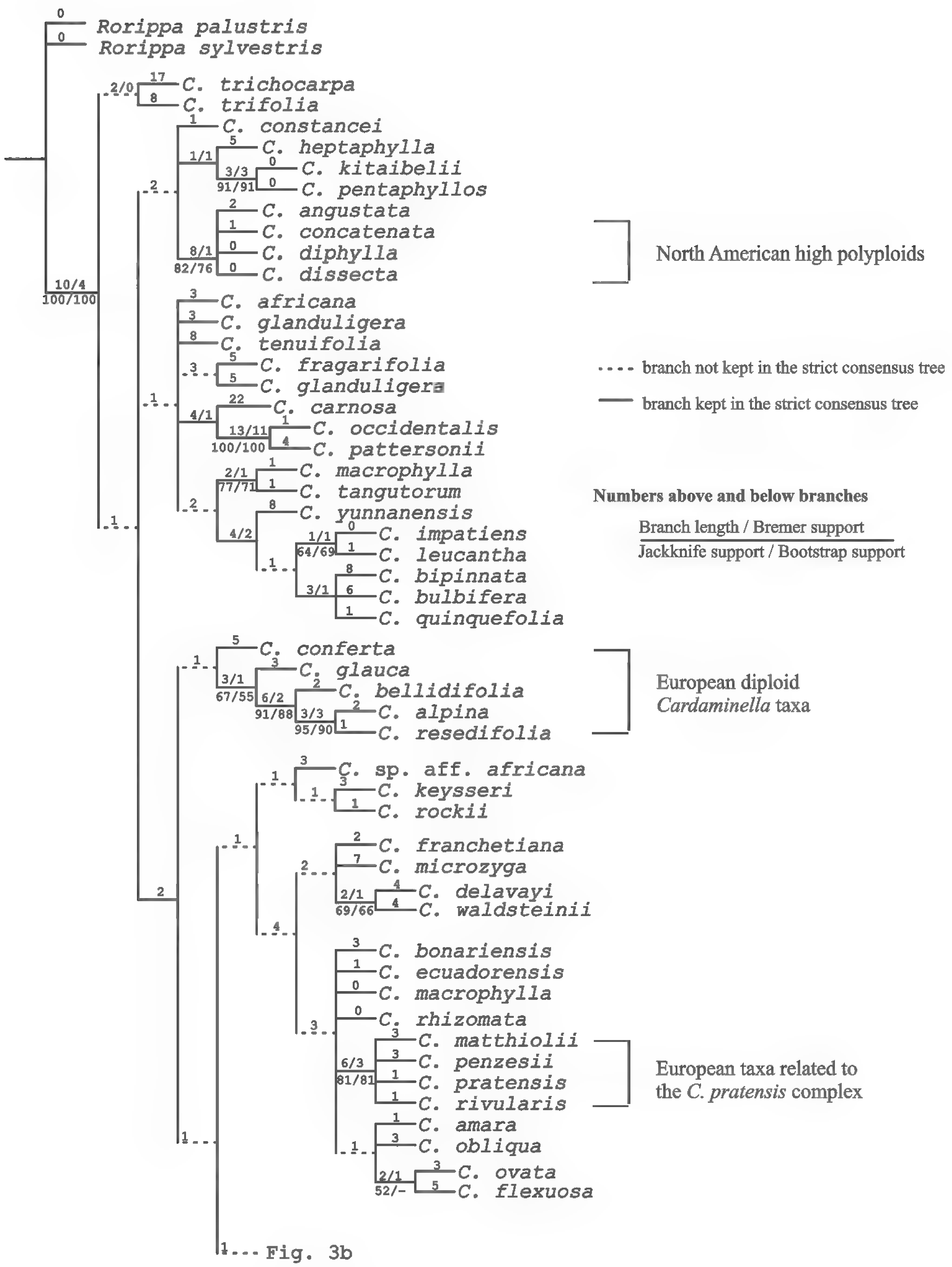

Figure 3. One of the MPTs based on the *trnL-F* data set showing branches kept in the strict consensus tree. Numbers above branches are Bremer support values. Numbers below branches are jackknife/bootstrap support values.

explanation for the lack of resolution is, therefore, that the initial diversification in *Cardamine* occurred rapidly.

Polyploidization, a common mode of evolution in *Cardamine* as well as in the Brassicaceae in general (Al-Shehbaz, 1988; Kucera et al., 2005; Beilstein et al., 2006; Marhold & Lihova, 2006), also could have affected the resolution of the ITS phylogeny. Our results imply that polyploidization has happened independently in many lineages in *Cardamine*. We

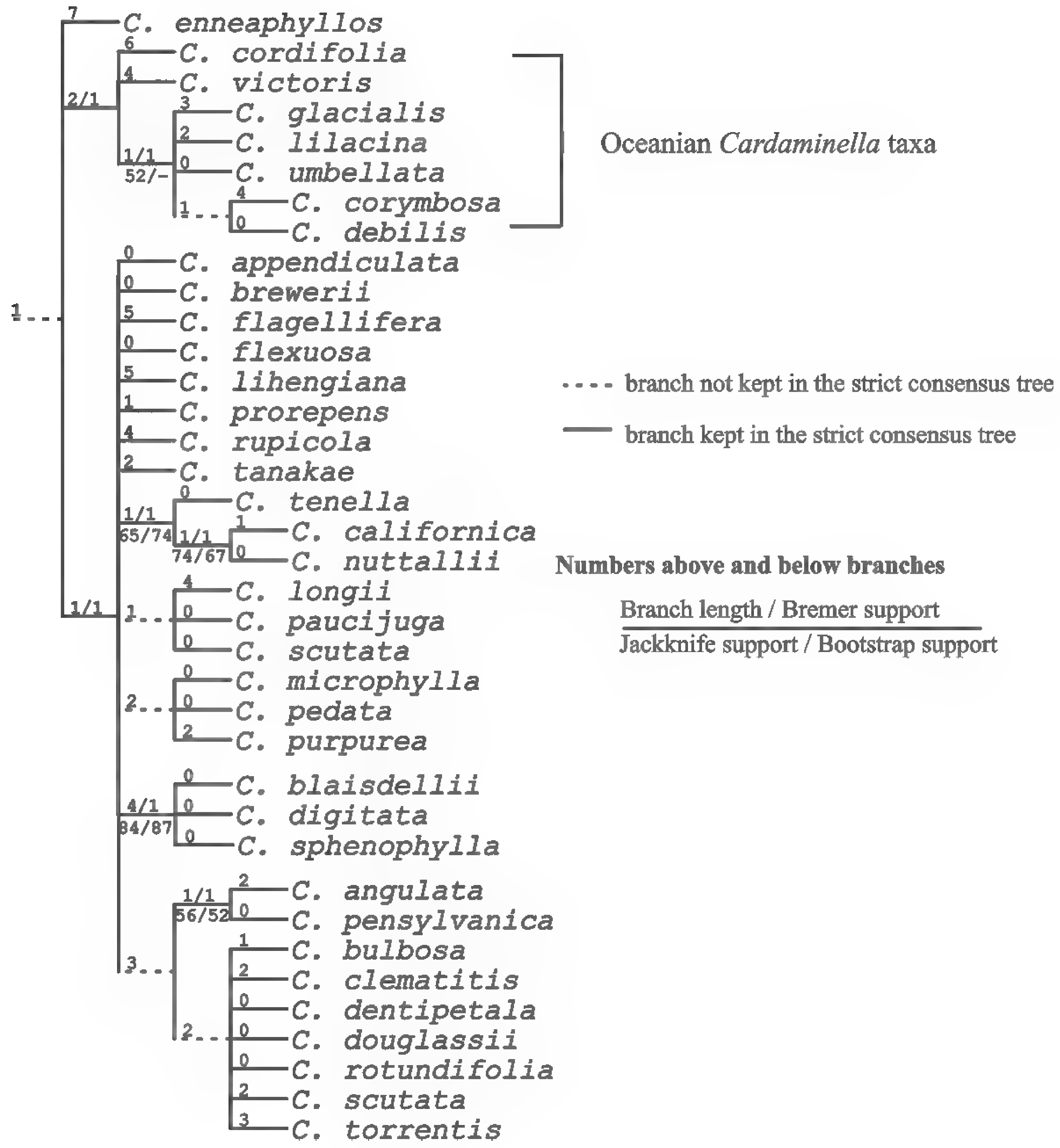

Figure 3. Continued.

found that nine of the 10 groups (noted A–J in Results and Figs. 1 and 2) contain diploid species, that only one group is exclusively diploid (group A), and that most groups contain species that are hexaploid or even higher. However, our separate analyses of the ITS region for diploids and tetraploids did not provide better resolution or conflicting topologies, nor did the plastid tree show better resolution. These results support our hypothesis that the lack of resolution is due to rapid speciation rather than breakdown of phylogenetic signal from frequent allopolyploidization.

Untangling the hierarchical structure among rapidly diverging lineages is difficult and requires a huge sequencing effort (Fishbein et al., 2001). We did an extensive survey of different regions in the preliminary analyses for this study, and a search for more phylogenetically informative molecular markers in *Cardamine* may prove fruitless.

Our results corroborate those of Koch et al. (2000) and Haubold and Wiehe (2001), who used molecular dating to demonstrate that *Cardamine* is a relatively young genus. After the split between *Cardamine* and *Barbarea* as late as 6.2 Ma (2–8 Ma), *Cardamine* rapidly diversified into one of the most species-rich genera in the Brassicaceae. This is consistent with the pattern observed in other large genera of this family, such as *Lepidium* L. (2.1–4.2 Ma, ca. 175 species; Mummenhoff et al., 2001) and *Draba* L. (4.5–9 Ma, ca. 350 species; Koch & Al-Shehbaz, 2002).

Several papers have addressed the importance and previous underestimation of long-distance dispersals to explain biogeographic patterns (Donoghue & Smith, 2004; Givnish & Renner, 2004; Thorne, 2004; Cook & Crisp, 2005; McGlone, 2005; Queiroz, 2005; Graham, 2006). In *Cardamine*, continental drift causing vicariant speciation can be ruled out because the present constellation of continents was established millions of years before the origin of the genus. Based on the estimate of 2–8 Ma, it is likely that the large late Tertiary forest in the Northern Hemisphere (Lafontaine & Wood, 1988; Bennike & Böcher, 1990; Matthews & Ovenden, 1990; Murray, 1995; Graham, 1999) provided the first habitat for establishment, divergence, and spread of *Cardamine*. It is possible that the later submerging of the Bering Land Bridge (Gladenkov et al., 2002) and the successive cooling of the Holarctic region (Zachos et al., 2001) has caused vicariant speciation in the genus. Despite its low overall resolution, our phylogeny nevertheless provides evidence for several extensive long-distance dispersal events (further discussed below).

SECTIONAL PARTITIONING

We found no support for monophyly of any of Schulz's (1903, 1936) large sections (Figs. 1–3). Section *Cardaminella* was not monophyletic; the species of this section occurred in different supported clades intermingled with species of other sections (compare clades in groups A, E, and J; Figs. 1, 2). Our results also reject the monophyly of section *Dentaria*, in agreement with Franzke et al. (1998) and Sweeney and Price (2000). Furthermore, the species included in the largest section, *Cardamine*, are spread among different supported groups and intermingled with species of other sections (e.g., groups G and J). This section has apparently served to include species that did not fit morphologically into any of the other 12 sections, as suggested by Sweeney and Price (2000). We have also shown that *Macrophyllum* and *Papyrophyllum* are not monophyletic, in agreement with Bleeker et al. (2002) and Sweeney and Price (2000; cf. Figs. 1–3).

RAPID COLONIZATION AND SUBSEQUENT DISPERSALS

Because of its poor resolution, our phylogeny was not suitable for biogeographic analyses such as DIVA to reconstruct ancestral areas (Ronquist, 1997). However, because of the considerably higher diversity of species and, in particular, diploid ones, Eurasia is certainly the most likely area of origin of *Cardamine*. Despite its typically short-distance main mode of dispersal, we hypothesize that the genus rapidly colonized and diversified in the entire Northern Hemisphere (Asia, Europe, and North America). Then, at a later stage, it spread across vast distances to the Southern Hemisphere as several distinct lineages (cf. also Bleeker et al., 2002). Both Eurasia and North America are likely source areas for dispersals to the Southern Hemisphere. Some distinct colonization episodes can be inferred based on supported groupings in our phylogeny and are shown in Figure 4.

Oceania. One example of very long-distance colonization (arrow f in Fig. 4) followed by rapid speciation is provided by the Australian and New Zealand taxa, which form a monophyletic group together with one Beringian (*Cardamine umbellata*) as well as one South American species (*C. glacialis*; subclade in J, Figs. 1, 2). Notably, these Oceanian species are morphologically diverse and comprise lowland as well as alpine taxa, but appear genetically similar. The four Oceanian species from New Guinea (*C.* sp. aff. *africana*, *C. altigena*, *C. keysseri*, and *C. papuana*), on the other hand, belonged to another distinct clade, which also comprised Northern Hemisphere taxa (group H; Figs 1, 2). Thus, Oceania appears to have been colonized at least twice from the Northern Hemisphere.

South America. We can discern at least four different dispersals into South America, two of which might originate from other Southern Hemisphere regions. We have shown that the accessions of *Cardamine africana* are monophyletic only by the inclusion of the South American *C. ovata*, which were suggested to be conspecific with *C. africana* earlier (Sjöstedt, 1975) (see subclade in group G). This must represent an independent dispersal event into South America, either from Africa or from the Northern Hemisphere with later dispersal to Africa (arrow d in Fig. 4). Long-distance dispersal from Africa to South America is not an impossible scenario (Graham, 2006).

As noted above, the South American *Cardamine glacialis* is most closely related to the species from Australia, Tasmania, and New Zealand in both the *trnL-F* (JK – 52%, BR – 1) and ITS (JK – 55%, BS – 57%, BR – 1, PP – 0.98) tree, and must represent a separate dispersal event into South America (arrow g in Fig. 4; subclade in group J).

The South American *Cardamine flaccida* and *C. bonariensis*, suggested by Sjöstedt (1975) to be conspecific, form a clade with the European *C. pratensis* complex and thus provide an example of yet another dispersal event (arrow c in Fig. 4; group I in Figs. 1, 2). From the parsimony ITS tree, the direction is impossible to determine as both ways are equally parsimonious (Cook & Crisp, 2005). We can infer either two dispersal events into South America or one old dispersal event to South America with a subsequent speciation event and then dispersal back to Europe.

Evidence for a fourth dispersal event into South America is provided by the sister group relationship (PP – 0.78, BR – 1) between the Asian *Cardamine griffithii* and the South American *C. ecuadorensis* and *C. rhizomata*, which form a separate clade within group F and may have originated from Eurasia (not indicated in Fig. 4). Our results reject Sjöstedt's (1975) hypothesis that *C. ecuadorensis* and *C. rhizomata* are conspecific with *C. africana*.

Africa. The African species did not form a monophyletic group in our analyses. The sister group of the African *Cardamine obliqua* differs between the ITS tree (group F) and the plastid tree, as also found by Bleeker et al. (2002), but it is possible that it has a

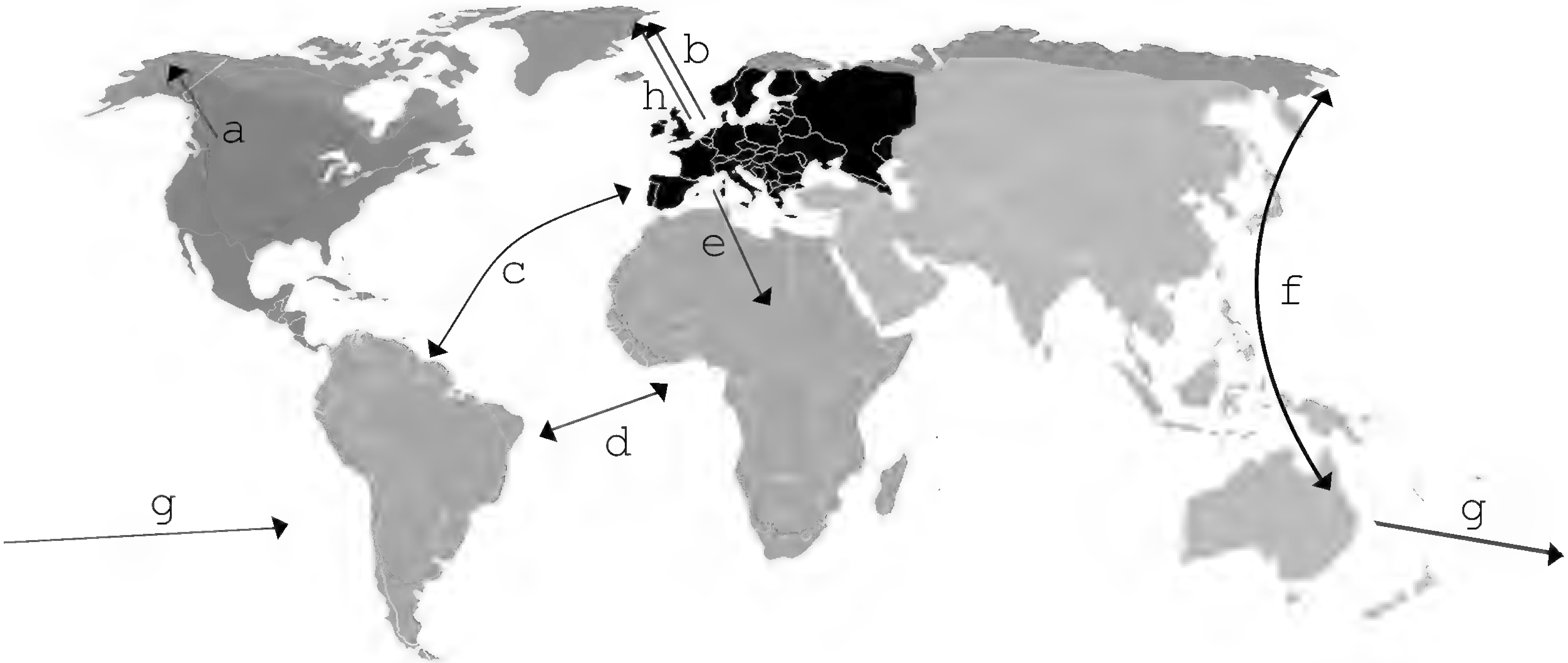

Figure 4. Map defining areas as used in the text and in Figures 1 and 2. Arrows indicate inferred major dispersal events discussed in the text (marked a–h).

Eurasian origin (arrow e in Fig. 4). The African *C. trichocarpa* (see group B) is highly divergent with its 15 ITS and 17 *trnL-F* autapomorphies and has no unambiguous sister group. It is not possible to infer its origin except that it is not sister to the other African taxa. Finally, *C. africana*, which occurs both in Africa and South America (cf. above), is member of yet another clade, nested with European and Asian taxa (group G; PP = 0.98 and BR = 1).

The Arctic. We can infer at least three major colonization episodes into the Beringian/circumpolar region. Many species of *Cardamine* reach the Arctic (e.g., *C. macrophylla*, *C. conferta* Jurtzev, *C. tenuifolia*, *C. prorepens* Fisch. ex DC., *C. scutata*, and *C. amara* L.), but here we focus on the nine taxa having the major part of their distribution in this region (*C. bellidifolia*, *C. blaisdellii*, *C. digitata*, *C. pratensis*, *C. purpurea*, *C. pedata*, *C. microphylla*, *C. victoris*, and *C. sphenophylla*).

Both the chloroplast and nuclear data demonstrate that the broadly circumpolar diploid *Cardamine bellidifolia* in group A is sister (JK = 77%, BS = 70%, PP = 1.0, and BR = 3 in ITS and JK = 91%, BS = 88%, and BR = 2 in *trnL-F*) to European diploids, specifically in the Alps and Pyrenees (arrow b in Fig. 4). This clade is sister to other European Mediterranean–Alpine diploids such as *C. glauca* in the *trnL-F* tree (JK = 67%, BS = 55%, and BR = 1) and *C. carnosa* and *C. plumieri* in the ITS tree (PP = 0.96 and BR = 1).

Another example of European origin is provided by the Arctic circumpolar *Cardamine pratensis* (group I). The Arctic specimens are regarded as a separate subspecies, *C. pratensis* subsp. *angustifolia* (Hook.) O. E. Schulz, which some authors regard as a separate species, *C. nymanii* Gand. (Franzke & Mummenhoff, 1999). *Cardamine pratensis* is a common boreal polyploid and belongs to a complicated species complex with many described diploids and low polyploids distributed throughout Europe. Our results are consistent with those of Franzke and Hurka (2000), who concluded that *C. pratensis* colonized the Arctic from southern Europe during the Holocene (arrow h in Fig. 4).

The remaining seven Arctic species (group J), which are restricted to the amphi-Beringian region, may have originated from one or two colonization events from North America (arrow a in Fig. 4), as these taxa are nested with other North American species. However, this is only inferred from the MPTs and the combinable components tree. Because we use collapsing rules that do not allow zero length branches, we did not get support for this hypothesis in the resampling analyses.

Thus, there are two distinct examples of European origin of Arctic *Cardamine*, including two different species that have become broadly distributed in the Arctic without further diversification. In addition, there is one example of a probable North American origin followed by diversification into many species in Beringia, but without further expansion into the circumarctic.

Literature Cited

Al-Shehbaz, I. A. 1988. The genera of Arabideae (Cruciferae, Brassicaceae) in the southeastern United States. J. Arnold Arb. 69: 85–166.

———, M. A. Beilstein & E. A. Kellogg. 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): An overview. Pl. Syst. Evol. 259: 89–120.

Beilstein, M. A., I. A. Al-Shehbaz & E. A. Kellogg. 2006. Brassicaceae phylogeny and trichome evolution. Amer. J. Bot. 93: 607–619.

Bennike, O. & J. Böcher. 1990. Forest-tundra neighbouring the North Pole—Plant and insect remains from the Plio-Pleistocene Kap København formation, North Greenland. Arctic 43: 331–338.

Bleeker, W. & H. Hurka. 2001. Introgressive hybridization in *Rorippa* (Brassicaceae): Gene flow and its consequences in natural and anthropogenic habitats. Molec. Ecol. 10: 2013–2022.

———, A. Franzke, K. Pollmann, A. H. D. Brown & H. Hurka. 2002. Phylogeny and biogeography of Southern Hemisphere high-mountain *Cardamine* species (Brassicaceae). Austral. Syst. Bot. 15: 575–581.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10: 295–304.

Cook, L. G. & M. D. Crisp. 2005. Directional asymmetry of long-distance dispersal and colonization could mislead reconstructions of biogeography. J. Biogeogr. 32: 741–754.

Donoghue, M. J. & S. A. Smith. 2004. Patterns in the assembly of temperate forests around the Northern Hemisphere. Philos. Trans., Ser. B 359: 1633–1644.

Elven, R., D. F. Murray, V. Y. Razzhivin & B. A. Yurtsev. 2006. Panarctic Flora Checklist. Vascular Plants Draft version (2006). National Centre for Biosystematics, University of Oslo, Oslo. <http://www.binran.ru/infsys/paflist/index.htm>, accessed 16 April 2009.

Farris, J. S. 1989. The retention index and the rescaled consistency index. Cladistics 5: 417–419.

———, V. A. Albert, M. Källersjö, D. Lipscomb & A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12: 99–124.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 4: 783–791.

Fishbein, M., C. Hibsch-Jetter, D. Soltis & L. Hufford. 2001. Phylogeny of Saxifragales (angiosperms, eudicots): Analysis of a rapid, ancient radiation. Syst. Biol. 50: 817–847.

Franzke, A. & K. Mummenhoff. 1999. Recent hybrid speciation in *Cardamine* (Brassicaceae)—Conversion of nuclear ribosomal ITS sequences in statu nascendi. Theor. Appl. Genet. 98: 831–834.

——— & H. Hurka. 2000. Molecular systematics and biogeography of the *Cardamine pratensis* complex (Brassicaceae). Pl. Syst. Evol. 224: 213–234.

———, K. Pollmann, W. Bleeker, R. Kohrt & H. Hurka. 1998. Molecular systematics of *Cardamine* and allied genera (Brassicaceae): ITS and non-coding chloroplast DNA. Folia Geobot. 33: 225–240.

Givnish, T. J. & S. S. Renner. 2004. Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreotropics, and transoceanic dispersal. Int. J. Pl. Sci. 165: S1–S6.

Gladenkov, A. Y., A. E. Oleinik, J. L. Marincovich & K. B. Barinov. 2002. A refined age for the earliest opening of Bering Strait. Palaeogeogr. Palaeoclimatol. Palaeoecol. 183: 321–328.

Goloboff, P. A. 1993. Estimating character weights during tree search. Cladistics 9: 83–91.

———, J. Farris & K. Nixon. 2003. TNT (Tree analysis using New Technology). Published by the authors, Tucumán, Argentina.

Graham, A. 1999. Late Cretaceous and Cenozoic History of North American Vegetation North of Mexico. Oxford University Press, New York.

———. 2006. Modern processes and historical factors in the origin of the African element in Latin America. Ann. Missouri Bot. Gard. 93: 335–339.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41: 95–98.

Harriman, N. A. 1965. The Genus *Dentaria* (Cruciferae) in Eastern North America. Ph.D. Dissertation, Vanderbilt University, Nashville.

Haubold, B. & T. Wiehe. 2001. Statistics of divergence times. Molec. Biol. Evol. 18: 1157–1160.

Heads, M. 2005. Dating nodes on molecular phylogenies: A critique of molecular biogeography. Cladistics 21: 62–78.

Hewson, H. J. 1982. Flora of Australia. Australian Government Publishing Service, Canberra.

Hofacker, I. L., W. Fontana, P. F. Stadler, S. Bonhoeffer, M. Tacker & P. Schuster. 1994. Fast folding and comparison of RNA secondary structures. Monatsh. Chem. 125: 167–188.

Huelsenbeck, J. P. & F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Kimata, M. 1983. Comparative studies on the reproductive systems of *Cardamine flexuosa*, *Cardamine impatiens*, *Cardamine scutata*, and *Cardamine lyrata*, Cruciferae. Bot. Mag. (Tokyo) 96: 299–312.

Kluge, A. G. & J. S. Farris. 1969. Quantitative phyletics and evolution of Anurans. Syst. Zool. 18: 1–32.

Koch, M. & I. A. Al-Shehbaz. 2002. Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). Ann. Missouri Bot. Gard. 89: 88–109.

———, B. Haubold & T. Mitchell-Olds. 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). Molec. Biol. Evol. 17: 1483–1498.

———, ——— & ———. 2001. Molecular systematics of the Brassicaceae: Evidence from coding plastidic *mat*K and nuclear *Chs* sequences. Amer. J. Bot. 88: 534–544.

———, C. Dobes, M. Matschinger, W. Bleeker, J. Vogel, M. Kiefer & T. Mitchell-Olds. 2005. Evolution of the *trn*F (GAA) gene in *Arabidopsis* relatives and the Brassicaceae family: Monophyletic origin and subsequent diversification of a plastidic pseudogene. Molec. Biol. Evol. 22: 1032–1043.

Kucera, J., I. Valko & K. Marhold. 2005. On-line database of the chromosome numbers of the genus *Cardamine* (Brassicaceae). Biologia 60: 473–476.

Lafontaine, J. D. & D. M. Wood. 1988. A zoogeographic analysis of the Noctuidae (Lepidoptera) of Beringia, and some inferences about past Beringian habitats. Mem. Entomol. Soc. Canad. 144: 109–123.

Lear, C. H., H. Elderfield & P. A. Wilson. 2000. Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite. Science 287: 269–272.

Lihova, J. & K. Marhold. 2003. Taxonomy and distribution of the *Cardamine pratensis* group (Brassicaceae) in Slovenia. Phyton (Horn) 43: 241–261.

———, J. Fuertes Aguilar, K. Marhold & G. Nieto Feliner. 2004. Origin of the disjunct tetraploid *Cardamine amporitana* (Brassicaceae) assessed with nuclear and chloroplast DNA sequence data. Amer. J. Bot. 91: 1231–1242.

Mai, D. H. 1995. Tertiäre Vegetationsgeschichte Europas. Gustav Fischer Verlag, Jena.

Marhold, K. & M. E. Ancev. 1999. *Cardamine penzesii*, a rediscovered taxon of the *C. pratensis* group (Cruciferae). Ann. Bot. Fenn. 36: 171–180.

——— & J. Lihova. 2006. Polyploidy, hybridization and reticulate evolution: Lessons from the Brassicaceae. Pl. Syst. Evol. V259: 143–174.

———, ———, M. Perny, R. Grupe & B. Neuffer. 2002. Natural hybridization in *Cardamine* (Brassicaceae) in the Pyrenees: Evidence from morphological and molecular data. Bot. J. Linn. Soc. 139: 275–294.

———, ———, ——— & W. Bleeker. 2004. Comparative ITS and AFLP analysis of diploid *Cardamine* (Brassicaceae) taxa from closely related polyploid complexes. Ann. Bot. (Oxford) 93: 507–520.

Marincovich, L. 2001. New evidence for the age of Bering Strait. Quatern. Sci. Rev. 20: 329–335.

——— & A. Y. Gladenkov. 1999. Evidence for an early opening of the Bering Strait. Nature 397: 149–151.

Matthews, J. V. & L. E. Ovenden. 1990. Late Tertiary plant macrofossils from localities in Arctic sub-arctic North America—A review of the data. Arctic 43: 364–392.

McGlone, M. S. 2005. Goodbye Gondwana. J. Biogeogr. 32: 739–740.

Mitchell, A. D. & P. B. Heenan. 2000. Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. Syst. Bot. 25: 98–105.

Mummenhoff, K., H. Bruggemann & J. L. Bowman. 2001. Chloroplast DNA phylogeny and biogeography of *Lepidium* (Brassicaceae). Amer. J. Bot. 88: 2051–2063.

Murray, D. F. 1995. Causes of Arctic plant diversity: Origin and evolution. Ecol. Stud. 113: 21–32.

Nylander, J. A. A. 2004. MrAIC.pl. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Queiroz, A. de. 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20: 68–73.

Rashid, A. & H. Ohba. 1993. A revision of *Cardamine loxostemonoides* O. E. Schulz (Cruciferae). J. Japan. Bot. 68: 199–208.

Ronquist, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Syst. Biol. 46: 195–203.

——— & J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Schulz, O. E. 1903. Monographie der Gattung *Cardamine*. Bot. Jahrb. Syst. 32: 280–623.

———. 1936. Cruciferae. Pp. 227–658 *in* A. Engler & H. Harms (editors), Die natürlichen Pflanzenfamilien. Engelmann, Leipzig.

Siebert, S. & R. Backofen. 2005. MARNA: Multiple alignment and consensus structure prediction of RNAs based on sequence structure comparisons. Bioinformatics 21: 3352–3359.

Simmons, M. P. & H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49: 369–381.

Sjöstedt, B. 1975. Revision of genus *Cardamine* L. (Cruciferae) in South America and Central America. Bot. Not. 128: 8–19.

Sweeney, P. W. & R. A. Price. 2000. Polyphyly of the genus *Dentaria* (Brassicaceae): Evidence from *trn*L intron and *ndh*F sequence data. Syst. Bot. 25: 468–478.

Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. Pl. Molec. Biol. 17: 1105–1109.

Thorne, R. 2004. Tropical plant disjunctions: A personal reflection. Int. J. Pl. Sci. 165: S137–S138.

Warwick, S. I. & I. A. Al-Shehbaz. 2006. Brassicaceae: Chromosome number index and database on CD-Rom. Pl. Syst. Evol. 259: 237–248.

Webb, C. J., W. R. Sykes & P. J. Garnock-Jones. 1988. Flora of New Zealand. Botany Division, Department of Scientific and Industrial Research, Christchurch.

White, T., T. Bruns, S. Lee & J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (editors), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.

Yang, Y. W., K. N. Lai, P. Y. Tai, D. P. Ma & W. H. Li. 1999. Molecular phylogenetic studies of *Brassica*, *Rorippa*, *Arabidopsis*, and allied genera based on the internal transcribed spacer region of 18S-25S rDNA. Molec. Phylogenet. Evol. 13: 455–462.

Zachos, J., M. Pagani, L. Sloan, E. Thomas & K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292: 686–693.

Appendix 1. Specimens included in this study. Section is given for species where it has been applied. Chromosome counts in parentheses refer to rare reports for species where multiple counts have been performed. Sequences taken from GenBank are listed with reference numbers and the corresponding publication. GenBank accession numbers for sequences produced for this publication are listed for each species and under each DNA region.

Species	Section	2*n* =	Country/Territory (Herbarium) Collector/Determinator[a]	ITS	*trnL* intron	*trnL-F* spacer
Rorippa palustris (L.) Bess.		32	Germany, Franzke et al., 1998; Bleeker & Hurka, 2001	AF078021, AF078022	AF079351	AF363669
R. sylvestris (L.) Bess.		48	Germany, Franzke et al., 1998; Bleeker & Hurka, 2001	AF078023, AF078024	AF079352	AF362644
Cardamine abchasica Govaerts	*Dentaria*		Georgia (WU)	FJ464476, FJ464499		
C. acris Griseb.	*Cardamine*	16	Montenegro, Marhold et al., 2004	AY245977, AY246007		
C. acris	*Cardamine*	16	Greece, Marhold et al., 2004	AY246002, AY246032		
C. sp. aff. *africana* L.[b]	*Papyrophyllum*	16	Papua New Guinea, Franzke et al., 1998; Bleeker et al., 2002	AF078009, AF078010	AF079342	AY047650
C. africana L. #1	*Papyrophyllum*	16	Tanzania, Bleeker et al., 2002	AY047612, AY047623	AY047639	AY047655
C. africana #2	*Papyrophyllum*	16	Ecuador, Bleeker et al., 2002	AY047611, AY047622	AY047642	AY047658
C. alpina Willd.	*Cardaminella*	16	Italy (OSBU), 223	FJ464463, FJ464486	FJ464509	FJ464529
C. altigena Schltr. ex O. E. Schulz			Papua New Guinea, Franzke et al., 1998	AF078011, AF078012	AF079343	
C. amara L. subsp. *amara*	*Cardamine*	16	Slovakia, Marhold et al., 2004	AY245985, AY246015		
C. amara subsp. *amara*	*Cardamine*	16	Italy, Lihova et al., 2004	AY260579		
C. amara subsp. *pyrenaea* Sennen	*Cardamine*	16	Spain, Franzke & Hurka, 2000		AF266633	
C. amara subsp. *pyrenaea*	*Cardamine*	16	Spain, Lihova et al., 2004	AY260580		
C. amara	*Cardamine*	16	Norway (O), Wesenberg		EU819147	
C. amporitana Sennen & Pau	*Cardamine*	32	Spain, Lihova et al., 2004	AY260585		
C. amporitana	*Cardamine*	32	Italy, Lihova et al., 2004	AY260608		
C. angulata Hook.	*Macrophyllum*	40	U.S.A., Washington State (MO), Thysell	FJ464464, FJ464487	FJ464522	FJ464545
C. angulata	*Macrophyllum*	40	U.S.A. (S), Calder, Savile & Taylor		EU819198	EU819240
C. angustata O. E. Schulz	*Dentaria*	128	U.S.A. (MO), Kral	FJ464465, FJ464488	FJ464520	FJ464542
C. angustata	*Dentaria*	128	U.S.A., Sweeney & Price, 2000		AF198121	
C. appendiculata Franch. & Sav.	*Macrophyllum*		Japan (O), H. Ogura	EU819308		
C. arisanensis Hayata			Taiwan (S)	EU819335	EU819199	
C. asarifolia L.	*Cardamine*	48	Italy, Lihova et al., 2004	AY260620		
C. barbaraeoides Halácsy	*Cardamine*	32	Greece, Lihova et al., 2004	AY260614		
C. bellidifolia L.	*Cardaminella*	16	Norway, Sweeney & Price, 2000		AF198122	
C. bellidifolia	*Cardaminella*	16	Spitsbergen (O), Elven		EU819148	EU819306

APPENDIX 1. Continued.

Species	Section	2*n* −	Country/Territory (Herbarium) Collector/Determinator[a]	ITS	*trnL* intron	*trnL-F* spacer
C. bellidifolia	*Cardaminella*	16	Russia (O), Maksimova	EU819310	EU819149	EU819305
C. bipinnata O. E. Schulz	*Dentaria*		Russia (WU)	FJ464466, FJ464489	FJ464513	FJ464535
C. blaisdellii Eastw.	*Cardaminella*	28, 42	U.S.A. (O), R. Elven	EU819313	EU819150	EU819301
C. blaisdellii	*Cardaminella*	28, 42	U.S.A. (O), R. Elven	EU819312	EU819151	EU819303
C. bonariensis Pers.	*Cardamine*		Ecuador (S), I. A. Al-Shehbaz	EU819314	EU819200	
C. bonariensis	*Cardamine*		Peru (MO), Duncan			EU819241
C. bradei O. E. Schulz			Costa Rica (S), R. L. Liesner	EU819318		
C. breweri S. Watson	*Cardamine*	84–96	U.S.A. (S), C. L. Porter & M. W. Porter	EU819315	EU819201	
C. breweri	*Cardamine*	84–96	U.S.A. (GA), Price		AF198123	
C. bulbifera Crantz	*Dentaria*	96	Germany, Franzke et al., 1998	AF078017, AF078018	AF079346	
C. bulbifera	*Dentaria*	96	Ukraine (MW)			FJ464534
C. bulbosa (Schreb. ex Muhl.) Britton, Sterns & Poggenb.		32–64	U.S.A. (MO), Kral		FJ464524	FJ464546
C. bulbosa		32–64	Canada (S), Gilles Lemieux	EU819316	EU819202	EU819242
C. bulbosa		32–64	U.S.A. (O), B. O. Wolden		EU819153	
C. bulbosa		32–64	U.S.A., Sweeney & Price, 2000		AF198124	
C. californica (Nutt.) Greene	*Eutreptophyllum*	32	U.S.A. (OSBU), Hurka	FJ464467, FJ464490	FJ464525	FJ464547
C. carnosa Waldst. & Kit.	*Pteroneurum*		Greece (S), K. H. Rechinger	EU819317	EU819203	EU819243
C. castellana Lihova & Marhold		16	Spain, Lihova et al., 2004	AY260578		
C. chelidonia L.	*Spirobolus*	64	Italy (O), J. Poelt		EU819154	
C. clematitis Shuttlew. ex S. Watson	*Cardamine*		U.S.A. (S), Leonard & Radford	EU819318	EU819204	EU819246
C. concatenata (Michx.) O. Schwarz	*Dentaria*	256	Canada (S), D. E. Swales	EU819319		
C. concatenata	*Dentaria*	256	Canada (WU)	EU819320		
C. concatenata	*Dentaria*	256	Canada (WU)		FJ464521	FJ464543
C. conferta Jurtzev		48	Russia (LE), L. Fokina	EU819321	EU819155, EU819156	EU819288, EU819291
C. constancei Detling			U.S.A. (S), R. C. Rollins	EU819322	EU819205	EU819244
C. cordifolia A. Gray	*Cardamine*	24	U.S.A. (S), Holmgren, Reveal & LaFrance	EU819324	EU819206	EU819245
C. cordifolia	*Cardamine*	24	U.S.A. (O), Dahl		EU819157	
C. corymbosa Hook. f.	*Cardaminella*	48	Australia, Bleeker et al., 2002	AF078003, AF078004	AF079339	AY047645
C. corymbosa	*Cardaminella*	48	New Zealand, Bleeker et al., 2002	AY047613, AY047624	AY047633	AY047646
C. crassifolia Pourr.		16	Spain, Lihova et al., 2004	AY260605		
C. debilis Banks ex DC.	*Cardamine*	48	New Zealand, Bleeker et al., 2002	AY047614, AY047625	AY047643	AY047660

APPENDIX 1. Continued.

Species	Section	2n =	Country/Territory (Herbarium) Collector/Determinator[a]	ITS	*trnL* intron	*trnL-F* spacer
C. delavayi Franch.			China (MO), 14549	EU819325		EU819248
C. densiflora Gontsch.			Tajikistan (MW)	FJ464468, FJ464491		
C. dentipetala Matsum.			Japan (S), M. Mizushima	EU819326	EU819207	
C. digitata Richardson	*Cardaminella*	28, 42	U.S.A. (O), Elven	EU819330	EU819158	
C. digitata	*Cardaminella*	28, 42	U.S.A. (O), Elven	EU819329	EU819159	EU819302
C. digitata	*Cardaminella*	28, 42	Canada (O), ver. by Elven	EU819327, EU819328	EU819160, EU819161	EU819304, EU819292
C. digitata	*Cardaminella*	28, 42	Russia (MW)	FJ464469, FJ464492		
C. digitata	*Cardaminella*	28, 42	Russia (MW)		FJ464512	FJ464533
C. diphylla (Michx.) Alph. Wood	*Dentaria*	96–256	Canada (S), Blondeau	EU819331	EU819208	EU819249
C. dissecta (Leavenw.) Al-Shehbaz	*Dentaria*		U.S.A. (WU)	FJ464470, FJ464493	FJ464528	FJ464544
C. douglassii Britton		28–72	U.S.A. (S), Hainault	EU819332	EU819209	EU819247
C. douglassii		28–72	Canada (O), Hainault		EU819162	
C. ecuadorensis Hieron.	*Cardamine*		Ecuador (S), Brekke	EU819333	EU819210	
C. enneaphyllos (L.) Cranz	*Dentaria*	80	Austria, Kärnten (OSBU), 5182	FJ464471, FJ464494	FJ464515	FJ464537
C. flaccida Cham. & Schltdl.	*Cardamine*	16	Chile (O), Skottsberg	EU819334		
C. flexuosa With.	*Cardamine*	32	Germany, Franzke et al., 1998; Bleeker et al., 2002	AF077999, AF077800	AF079337	AY047644
C. fragariifolia O. E. Schulz	*Cardamine*		China (MO), 317	EU819336		EU819250
C. franchetiana Diels			China (MO), 20021	EU819337		EU819251
C. gallaecica (M. Laínz) Rivas Mart. & Izco		(32) 48	Spain, Lihova et al., 2004	AY260613		
C. glacialis (G. Forst.) DC.	*Cardamine*	72	Chile, Bleeker et al., 2002	AY047615, AY047626	AY047634	AY047648
C. glacialis	*Cardamine*	72	Argentina, Bleeker et al., 2002	AY047616, AY047627	AY047635	AY047649
C. glanduligera O. Schwarz	*Dentaria*	42	Regensburg Botanical Garden (Germany)	EU819338	EU819163	
C. glanduligera	*Dentaria*		Ukraine (MW)		FJ464518	FJ464540
C. glanduligera	*Dentaria*		Russia (MW)		FJ464519	FJ464541
C. glauca Spreng.	*Pteroneurum*	16	Italy (S), Segelberg	EU819211		EU819253
C. graeca L.	*Pteroneurum*	16–18	Romania (O), Borza & Bujorean		EU819164	
C. griffithii Hook. f. & Thomson	*Cardamine*		China (MO), 496	EU819339		EU819254
C. heptaphylla (Vill.) O. E. Schulz	*Dentaria*	48	Italy (OSBU), Bernhardt	FJ464472, FJ464495	EU819212	EU819255
C. hirsuta L.	*Cardamine*	16	Germany, Franzke et al., 1998	AF077997, AF077998		
C. impatiens L.	*Cardamine*	16	Germany, Franzke et al., 1998	AF078015, AF078016		
C. impatiens	*Cardamine*	16	Norway (O), K. Lye		EU819167	

APPENDIX 1. Continued.

Species	Section	$2n$ —	Country/Territory (Herbarium) Collector/Determinator[a]	ITS	*trnL* intron	*trnL-F* spacer
C. keysseri O. E. Schulz			New Guinea, Franzke et al., 1998	AF078013, AF078014	AF079344	AY047651
C. kitaibelii Bech.	*Dentaria*		Bosnia-Herzegovina, (WU)	FJ464473, FJ464496	FJ464516	FJ464538
C. kitaibelii	*Dentaria*		Switzerland (S), Simon		EU819214	EU819257
C. laciniata (Muhlenb.) Wood	*Dentaria*		Canada (S), Swales		EU819215	
C. lacustris (Garn.-Jones & P. N. Johnson) Heenan		48	New Zealand, Mitchell & Heenan, 2000	AF100683		
C. leucantha O. E. Schulz	*Macrophyllum*	16	Japan (S), M. Mizushima		EU819216	EU819260
C. leucantha	*Macrophyllum*	16	Japan (S), M. Furuse	EU819340		
C. leucantha	*Macrophyllum*	16	Russia (MW)	FJ464474, FJ464497		
C. lihengiana Al-Shehbaz			China (MO), 1207	EU819342		EU819261
C. lilacina Hook.		48	Australia, Franzke et al., 1998	AF078007, AF078008	AF079341	AY047659
C. lilacina		48	Australia, Franzke et al., 1998	AF078005, AF078006	AF079340	AY047647
C. longii Fernald			U.S.A. (S), Fernald & Long	EU819343	EU819217	EU819262
C. lyallii S. Watson	*Cardamine*		U.S.A. (S), R. C. Rollins	EU819323		
C. macrophylla Willd.	*Macrophyllum*	64–96	Japan (MO), Al-Shehbaz	EU819345		EU819263
C. macrophylla	*Macrophyllum*	64–96	Russia (MW)	FJ464475, FJ464498		
C. matthioli Moretti ex Comolli	*Cardamine*	16	Slovenia, Lihova et al., 2004	AY260606		
C. matthioli	*Cardamine*	16	Bulgaria, Franzke & Hurka, 2000		AF266642	
C. matthioli	*Cardamine*	16	Slovakia, Franzke et al., 1998		AF079330	Af266597
C. microphylla Adams	*Cardaminella*	28–64	Russia (LE), Tolmachev & Polozova	EU819347	EU819172	
C. microphylla	*Cardaminella*	28[c]	Russia (LE), Zhukova	EU819346		
C. microphylla	*Cardaminella*	28–64	Russia (LE), Tolmachev & Yurtsev		EU819173	EU819290
C. microphylla	*Cardaminella*	28–64	Russia (O), Plyeva		EU819170	
C. microphylla	*Cardaminella*	28–64	Russia (MW)			FJ464532
C. microzyga O. E. Schulz	*Cardamine*		China (S), J. F. Rock	EU819348	EU819220	EU819266
C. nipponica Franch. & Sav.	*Cardaminella*		Japan (S), M. Tamura, 9141	EU819349		
C. nuttallii Greene	*Eutreptophyllum*		U.S.A., California (MO), Taylor	EU819350	FJ464523	EU819267
C. obliqua Hochst. ex A. Rich.	*Cardamine*	36–72	Kenya, Bleeker et al., 2002	AY047617, AY047628	AY047636	AY047652
C. obliqua	*Cardamine*	36–72	Ethiopia, Bleeker et al., 2002	AY047618, AY047629	AY047638	AY047654
C. obliqua	*Cardamine*	36–72	Kenya (S), Å. Strid		EU819223	
C. occidentalis (Watson) O. E. Schulz	*Cardamine*	64	U.S.A. (OSC), 335917	EU819351		EU819269
C. occidentalis	*Cardamine*	64	U.S.A. (OSC), 357322	EU819352		EU819268
C. ovata Benth.	*Papyrophyllum*		Ecuador (S), Al-Shehbaz	EU819353	EU819224	
C. ovata	*Papyrophyllum*		Ecuador (S), Al-Shehbaz		EU819225	

Appendix 1. Continued.

Species	Section	$2n$ –	Country/Territory (Herbarium) Collector/Determinator[a]	ITS	*trnL* intron	*trnL-F* spacer
C. ovata	*Papyrophyllum*		Ecuador (MO), Al-Shehbaz			EU819270
C. papuana O. E. Schulz	*Papyrophyllum*		Papua New Guinea (CANB), 13035	FJ464477, FJ464500		
C. parviflora L.	*Cardamine*	16	Slovakia (O), Cernoch	EU819354		
C. parviflora	*Cardamine*	16	Austria (OSBU), Hurka	FJ464478, FJ464501		
C. pattersonii L. F. Hend.			U.S.A. (OSC), 245394	EU819355		EU819271
C. paucijuga Turcz.			Australia, Bleeker et al., 2002	AY047619, AY047630	AY047640	AY047656
C. pedata Regel & Tiling	*Cardaminella*	30[c]	Russia (LE), Zhukova, Yurtsev & Petrovsky	EU819356	EU819175, EU819176	EU819276, EU819277
C. pensylvanica Muhl. ex Willd.		32, 64	U.S.A. (ALA), Duffy		EU819177	
C. pensylvanica		32, 64	U.S.A. (ALA), De Lapp & Duffy		EU819178	
C. pentaphyllos (L.) Crantz	*Dentaria*	48	V7030-2003 (Botanical Garden, Natural History Museum of Oslo)	EU819357		
C. penzesii Ančev & Marhold		16	Bulgaria, Franzke & Hurka, 2000		AF266643	AF266600
C. plumieri Vill.	*Cardaminella*	16	Italy (GAT)	FJ464479, FJ464502		
C. pratensis L.	*Cardamine*	16–118	Portugal, Marhold et al., 2004	AY245995, AY246025		
C. prorepens Fisch. ex DC.	*Cardamine*	18, 20	Russia (LE), Yurtsev		EU819179	
C. prorepens	*Cardamine*	18, 20	Russia (LE), A. P. Arkhangelskya		EU819180	
C. prorepens	*Cardamine*	18, 20	Russia (MW)	FJ464480, FJ464503		
C. purpurea Cham. & Schltdl.	*Cardaminella*	96[c]	Russia (LE), Petrovsky	EU819362	EU819185	
C. purpurea	*Cardaminella*	96[c]	Russia (LE), Petrovsky	EU819363	EU819186	EU819294
C. purpurea	*Cardaminella*	80	U.S.A. (O), Elven	EU819360	EU819181	
C. purpurea	*Cardaminella*	80	U.S.A. (O), Elven	EU819361	EU819182	EU819275
C. purpurea	*Cardaminella*	80	U.S.A. (O), Murray, Yurtzev, Kelso	EU819358	EU819183	EU819293
C. purpurea	*Cardaminella*	80	U.S.A. (O), Elven & Grundt	EU819359	EU819184	
C. purpurea	*Cardaminella*	80	Russia (MW)		FJ464511	FJ464531
C. quinquefolia (M. Bieb.) Benth. & Hook. f. ex Schmalh.	*Dentaria*		Ukraine (MW)	FJ464481, FJ464504	FJ464514	FJ464536
C. resedifolia L.	*Cardaminella*	16	Austria (S), Emanuelsson	EU819364	EU819228	EU819273
C. resedifolia	*Cardaminella*	16	Italy (OSBU), 123	FJ464482, FJ464504	FJ464510	FJ464530
C. rhizomata Rollins			Ecuador (S), Al-Shehbaz	EU819365	EU819229	
C. rivularis Schur		16	Romania, Franzke et al., 1998	AF077981, AF077982	AF079328	AF266595
C. rivularis		16	Bulgaria, Franzke & Hurka, 2000	AF265201, AF265181		
C. rockii O. E. Schulz	*Cardamine*		China (S), Rock	EU819366	EU819230	
C. rotundifolia Michx.	*Cardamine*		U.S.A. (S), Gilbert	EU819367	EU819231	EU819274

APPENDIX 1. Continued.

Species	Section	$2n$ —	Country/Territory (Herbarium) Collector/Determinator[a]	ITS	*trnL* intron	*trnL-F* spacer
C. rupicola (Schulz) Hitch.			U.S.A. (S), Cronquist	EU819368	EU819232	EU819278
C. scutata Thunb.		32	Japan (MO18034)	EU819372		EU819279
C. scutata		32	Taiwan (S), Hsu	EU819370	EU819227	EU819272
C. scutata var. *formosana* (Hayata) T. S. Liu & S. S. Ying		32	Taiwan, Yang et al., 1999	AF128107		
C. sphenophylla Jurtzev (isotype)	*Cardaminella*	28[c]	Russia (LE), Kozhevnikov & Yurtsev	EU819373	EU819187	
C. tanakae Franch. & Sav.	*Cardamine*		Japan (S), Miyoshi Furuse	EU819374	EU819233	EU819281
C. tanakae	*Cardamine*		Japan (MO), Kurosawa	EU819375		EU819280
C. tangutorum O. E. Schulz	*Dentaria*	42	China (S), Schulz	EU819376	EU819234	EU819282
C. tenella (Pursh) O. E. Schulz	*Eutreptophyllum*		U.S.A. (S), Cronquist		EU819235	
C. tenera S. G. Gmel. ex C. A. Mey.	*Cardamine*	16	Russia, Marhold et al., 2004	AY245980, AY246010		
C. tenuifolia (Ledeb.) Turcz.	*Sphaerotorrhiza*	32	Russia (S), Petrovsky & Plieva			EU819283
C. tenuifolia	*Sphaerotorrhiza*	32	Russia (MW)		FJ464527	FJ464549
C. torrentis Nakai		56	Japan (S), T. Yoshimura		EU819236	
C. torrentis		56	Japan (S), M. Mizushima	EU819377	EU819237	EU819284
C. trichocarpa Hochst. ex A. Rich.	*Cardamine*	16, 32	Ethiopia, Bleeker et al., 2002	AY047620, AY047631	AY047641	AY047657
C. trichocarpa	*Cardamine*	16, 32	Tanzania, Bleeker et al., 2002	AY047621, AY047632	AY047637	AY047653
C. trifolia L.	*Coriophyllum*	16	Austria (OSBU), Bernhardt	FJ464483, FJ464506	FJ464526	FJ464548
C. uliginosa M. Bieb.	*Cardamine*	16	Georgia, Marhold et al., 2004	AY245981, AY246011		
C. umbellata Greene	*Cardaminella*	32, 48	U.S.A. (O), Elven	EU819379	EU819189	EU819296
C. umbellata	*Cardaminella*	32, 48	U.S.A. (O), Elven	EU819380	EU819190	EU819297
C. umbellata	*Cardaminella*	32, 48	U.S.A. (O), Elven	EU819378	EU819191	EU819295
C. victoris N. Busch	*Cardaminella*	28[c]	Russia (LE), Kozhevnikov & Yurtsev	EU819383	EU819195	
C. victoris	*Cardaminella*	28[c]	Russia (LE), Yurtsev & Zhukova	EU819382	EU819194	EU819298
C. victoris	*Cardaminella*	28[c]	Russia (LE), Koropkov	EU819381	EU819193	EU819299
C. victoris	*Cardaminella*	28	Russia (O), ver. by R. Elven		EU819192	
C. waldsteinii Dyer	*Dentaria*		Bosnia-Herzegovina (WU)	FJ464484, FJ464507	FJ464517	FJ464539
C. waldsteinii	*Dentaria*		Sweeney & Price, 2000		AF198135	
C. waldsteinii	*Dentaria*		Austria (S), Teppner		EU819238	
C. waldsteinii	*Dentaria*		Austria (S), Korb		EU819213	EU819256
C. yezoensis Maxim.	*Macrophyllum*	32	Russia (MW)	FJ464485, FJ464508		
C. yunnanensis Franch.	*Cardamine*		China (MO), 12360	EU819384		EU819285

[a] Where sequences have been retrieved from GenBank, only country/territory and reference are given.

[b] This specimen was identified as *Cardamine africana*, but results from our study indicate that this specimen represents a separate taxon, termed *C.* sp. aff. *africana* L. in this paper.

[c] Indicates that our voucher for the DNA sample is the same voucher as for the chromosome count. Chromosome counts follow the compilations in Kucera et al. (2005) and Warwick and Al-Shehbaz (2006).